UNIVERSIDAD COMPLUTENSE DE MADRID

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Departamento de Genética

DELIMITACIÓN DE ESPECIES DEL GÉNERO *CLADONIA*: REVISIÓN Y EVALUACIÓN DE ESPECIES CONFLICTIVAS

MEMORIA PARA OPTAR AL GRADO DE DOCTOR
PRESENTADA POR

Raquel Pino Bodas

Bajo la dirección de las doctoras

Ana Rosa Burgaz
Mª Paz Martín

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DELIMITACIÓN DE ESPECIES EN EL GÉNERO *CLADONIA*: REVISIÓN Y EVALUACIÓN DE ESPECIES CONFLICTIVAS

RAQUEL PINO BODAS

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Dra. Ana Rosa Burgaz, catedrática de botánica del departamento de Biología Vegetal I de la U.C.M. y Dra. María Paz Martín, investigadora científica del Real Jardín Botánico de Madrid, CSIC.

Certifican,

Que la Lda. Raquel Pino Bodas ha realizado bajo nuestra dirección la tesis doctoral titulada: **Delimitación de especies en el género *Cladonia*: revisión y evaluación de especies conflictivas** y que cumple con los requisitos necesarios para aspirar al grado de doctor en biología por la Universidad Complutense de Madrid.

Vº Bº de la Directora

Ana Rosa Burgaz

Vº Bº de la Directora

Mª Paz Martín

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ÍNDICE

ABREVIATURAS

B: herbario de Berlín
BG: herbario de la Universidad de Bergen
BLAST: programa del NCBI utilizado para buscar la identidad de las secuencias (Basic Local Alignment Search Tool)
bp: pares de bases
BRA: herbario del Museo Nacional de Eslovaquia
BSA: albúmina de suero bovino (Bovine Serum Albumin)
CANB: herbario Nacional de Australia
cox1: citocromo c oxidasa
DNA: ácido desoxirribonucleico
ef1α: factor de elongación 1 alfa (elongation factor 1 alpha)
F: herbario del Field Museum, Chicago
FH: herbario de la Universidad de Harvard
FR: herbario del Museo de Historia Natural Senckenberg, Frankfurt
GDA: herbario de la Universidad de Granada
GAPDH: gliceraldehido 3-fosfato deshidrogenasa
H: herbario de la Universidad de Helsinki
ICEL: herbario del Instituto de Historia Natural de Islandia
IGS: espaciador intergénico (intergenic spacer)
ITS: espaciador transcrito interno (internal transcribed spacer)
K o KOH: test químico realizado con potasa
K2P: modelo evolutivo de sustitución nucleotídica Kimura dos parámetros
L: herbario Nacional de Holanda
MACB: herbario de la Facultad de Biología, Universidad Complutense, Madrid
MA-lichen: herbario del Real Jardín Botánico de Madrid, líquenes
ML: máxima verosimilitud (maximum likelihood)
MP: máxima parsimonia (maximum parsimony)
mtLSU: subunidad grande ribosomal del DNA mitocondrial (mitochondrial DNA large ribosomal subunit)
NCBI: Centro Nacional de Información Biotecnológica (National Center for Biotechnology Information)
PCR: reacción en cadena de la polimerasa (Polymerase chain reaction)
rDNA: DNA ribosómico
rpb1: gen codificante para la subunidad mayor de la RNA polimerasa II
rpb2: gen codificante para la segunda subunidad mayor de la RNA polimerasa II
S: herbario del Museo de Historia Natural de Suecia
SEM: microscopía electrónica de barrido (Scanning electron microscope)
TLC: cromatografía en capa fina (Thin layer chromatography)
UPS: herbario de la Universidad de Upsala
UV: luz ultravioleta

RESUMEN

El género de hongos liquenizados *Cladonia* está compuesto por más de 450 especies distribuidas a lo largo de todo el mundo. La mayoría de las especies presenta una gran variación fenotípica, lo que ha dificultado la delimitación de los taxones mediante caracteres morfológicos. En este trabajo se analiza el límite entre las especies en *Cladonia* mediante el reconocimiento filogenético de especie por concordancia genealógica. Para ello, se seleccionaron varios grupos de especies en los cuales los límites entre los taxones y la independencia de los mismos han sido controvertidos. Dichos grupos son: 1) *Cladonia convoluta* y *C. foliacea*; 2) *C. iberica* y *C. subturgida;* 3) *C. rei* y *C. subulata*; 4) el grupo de *C. cariosa*; 5) el grupo de *C. gracilis;* 6) el grupo de *C. humilis*; y 7) *C. furcata* y *C. subrangiformis*. En cada uno de estos grupos se ha determinado si los diferentes taxones constituyen linajes independientes, así como el valor que presentan para la delimitación de especies tanto los caracteres morfológicos como los metabolitos secundarios. Durante el desarrollo de esta tesis doctoral se han revisado las identificaciones de más de 3500 muestras, de las cuales se seleccionaron 469 especímenes para los estudios moleculares. Se han analizado 3 o 4 loci, siendo en todos los casos ITS rDNA y *rpb2* dos de los loci seleccionados. Otros loci seleccionados sobre la base de los ensayos iniciales fueron *cox1*, mtLSU, *ef1α* o IGS rDNA. En el caso de *C. convoluta* y *C. foliacea,* estos taxones no formaron grupos monofiléticos independientes, por lo que las diferencias morfológicas encontradas entre ambos taxones (en cuanto a tamaño del talo primario) podrían constituir una respuesta a las diferentes condiciones ambientales, tales como el tipo de sustrato y el grado de aridez. Tampoco los análisis filogenéticos separaron a *Cladonia iberica* y *C. subturgida* en dos grupos monofiléticos. En este caso, las diferencias morfológicas y químicas se han interpretado como mera variación intraspecífica.

Los especímenes de *C. turgida* var. *corsicana* formaron un grupo monofilético independiente de *C. turgida* s. str. Estos resultados son coherentes con las diferencias fenotípicas. Por tanto, se propone una nueva combinación, *C. corsicana*. Los análisis filogenéticos mostraron que *C. corsicana* está relacionada con especies de la antigua sección *Ascyphiferae.*

Las especies *C. subulata* y *C. rei*, muy semejantes en su morfología, formaron dos linajes filogenéticos independientes, cada uno de ellos asociado a un conjunto de caracteres fenotípicos distintos. Se han encontrado diferencias significativas en el tamaño de los soredios, el grosor de la pared del podecio, la presencia de escuámulas sobre los podecios y la ultraestructura del estereoma. Sin embargo, los metabolitos secundarios siguen siendo los caracteres más fiables para distinguir estas dos especies.

Los resultados filogenéticos mostraron que el grupo de *C. cariosa* está formado al menos por cuatro especies filogenéticas. Los cuatro clados agruparon a varios quimiótipos, algunos de ellos compartidos por varios clados, lo cual demuestra el limitado valor taxonómico de los metabolitos secundarios en este grupo.

El grupo de *C. gracilis* constituyó un grupo monofilético, aunque los taxones de dicho grupo no son monofiléticos, a excepción de *C. ecmocyna* y *C. cornuta* subsp. *cornuta*. *Cladonia coniocraea*, *C. ochrochlora* y *C. cornuta* subsp. *groenlandica* han resultado ser coespecíficas. La identidad de la mayor parte de los miembros de este grupo no ha podido ser resuelta debido a la falta de variación genética de los loci estudiados. Los caracteres morfológicos utilizados para la delimitación de especies en este grupo han resultado ser muy homoplásicos.

La filogenia del complejo de *C. humilis* ha revelado que no es un grupo monofilético, puesto que *C. nashii* no está estrechamente relacionada con el resto de taxones del complejo. Los especimenes de *C. humilis* se separan en dos clados monofiléticos que se corresponden con los dos quimiótipos descritos. Estos clados no están estrechamente relacionados y se consideran dos especies independientes (*C. humilis* y *C. conista*). Los especímenes de *C. hammeri* procedentes de Norteamérica forman un grupo monofilético y son genéticamente diferentes de los europeos. Los taxones con un tamaño similar de soredios, o con un mismo tipo de córtex, no están estrechamente relacionados.

Los análisis filogenéticos del complejo de *Cladonia furcata* y *C. subrangiformis* han revelado la existencia de dos clados monofiléticos. Sin embargo, ninguno de estos clados corresponde los taxones previamente descritos. Dichos clados son muy heterogéneos desde el punto de vista morfológico y químico.

Con todas las secuencias generadas en esta tesis doctoral se ha realizado un análisis de barcoding para determinar cuál de los genes empleados es el más idóneo para la identificación de especímenes de *Cladonia* mediante caracteres moleculares. A pesar de que ITS rDNA es el locus elegido como primer barcode en hongos, en el caso de *Cladonia* la tasa de éxito de identificación del locus *rpb2* fue del 100%.

ABSTRACT

The genus of lichenized fungi *Cladonia* comprises more than 450 species distributed the world over. Most of these species have a high phenotypical variation, what has made it difficult to delimit the diverse taxa by means of morphological characters. In this work, the limits among the species within *Cladonia* are analyzed using the genealogical concordance phylogenetic species recognition. To this end, several groups of species were selected, in which the independence of the taxa and the limits among them have been controversial. Such groups are 1) *Cladonia convoluta* and *C. foliacea*; 2) *C. iberica* and *C. subturgida;* 3) *C. rei* and *C. subulata*; 4) *C. cariosa* group; 5) *C. gracilis* group; 6) *C. humilis* group; and 7) *C. furcata* and *C. subrangiformis*. For each of these groups it was determined whether the different taxa are independent lineages; the value that both morphological characters and secondary metabolites have for species delimitation was also assessed. Over the progression of this doctoral thesis, the identifications of more than 3400 samples have been reviewed; 469 specimens of which were selected for molecular studies. Three or four loci were analized, ITS rDNA and *rpb2* being two of them in every case. Other loci selected on the base of the initial trials were *cox1*, mtLSU, *ef1α* and IGS rDNA. Regarding *C. convoluta* and *C. foliacea*, these taxa did not form independent monophyletic groups, whereby the morphological differences found between both taxa (concerning the size of the primary thallus) could be but a response to environmental conditions, such as the substrate type and the aridity degree. Phylogenetic analyses did not separate *Cladonia iberica* and *C. subturgida* neither into two monophyletic groups. In this case, the morphological and chemical differences have been interpreted as mere intraspecific variation.

The specimens of *C. turgida* var. *corsicana* did form a monophyletic group, independent from *C. turgida* s. str. These results are consistent with the phenotypical differences. Therefore, the new combination *C. corsicana* is proposed. Phylogenetic analyses proved that *C. corsicana* is related to species within the old section *Ascyphiferae.*

The species *C. subulata* and *C. rei,* very similar in their morphology, formed two independent phylogenetic lineages, each of them linked to a set of distinct phenotypical characters. Significant differences have been found as for the soredia size, podetial wall thickness, presence of squamules on podetia and stereome structure. However, secondary metabolites still are the most reliable characters in order to distinguish these species. Phylogenetic research proved that *C. cariosa* group is constituted by four phylogenetic species at least. The four clades grouped together several chemotypes, some of them shared by various clades, what is clear proof of the limited taxonomical value of secondary metabolites in this group.

Cladonia gracilis group turned out to be a monophyletic group, though the taxa within this group are not monophyletic, except *C. ecmocyna* and *C. cornuta* subsp. *cornuta*. *Cladonia coniocraea*, *C. ochrochlora* and *C. cornuta* subsp. *groenlandica* proved to be conspecific. The identity of most of the members of this group could not be solved due to the lack of genetic variation in the studied loci. The morphological characters used for species delimitation in this group are very homoplastic.

The phylogenetic study of *C. humilis* complex reveals that it is not a monophyletic group, since *C. nashii* is not closely related to the remaining taxa within the complex. *C. humilis* specimens separate into two monophyletic clades which correspond with both of the described chemotypes. These clades are not closely related and are

considered as two independent species (*C. humilis* and *C. conista*). The specimens of *C. hammeri* coming from North America form a monophyletic group and are genetically different from the European ones. The taxa with similar soredia size, or with the same cortex type, are not closely related.
The phylogenetic analyses of *Cladonia furcata* and *C. subrangiformis* have revealed the existence of two monophyletic clades. Nevertheless, none of these clades corresponds to the previously described taxa. These clades are highly heterogeneous from a morphological or chemical point of view. With all the sequences generated in this doctoral thesis, a barcoding analysis has been made in order to determine which of the genes used is the most suitable for specimens identification in *Cladonia* by means of molecular characters. Despite ITS rDNA being the chosen locus as first barcode in fungi, in the case of *Cladonia* the identification success rate of *rpb2* locus was 100%.

INTRODUCCIÓN

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Desde el fijismo tipológico de Linneo, cuestionado por él mismo en sus últimos años, pasando por el erróneamente fundado evolucionismo de Lamarck y el escepticismo de Darwin sobre el concepto de especie, este no ha dejado de mostrar aspectos controvertidos ni de resistirse a una definición universalmente aceptable hasta nuestros días. Según Mayden (1997), se han descrito hasta 22 diferentes conceptos de especie, siendo muy sutiles las diferencias entre algunos de ellos. Queiroz (1998) examinó las diferencias y similitudes entre los diferentes conceptos de especie y llegó a la conclusión de que todas las concepciones modernas de especie encierran una noción general en la que están tácitamente de acuerdo los biólogos actuales. Según esta noción, las especies son "segmentos de linajes evolutivos en el nivel de las poblaciones". Los diferentes conceptos de especie pueden clasificarse en cuatro grupos principales: el concepto fenotípico (o morfológico), el concepto biológico, el concepto filogenético y el concepto evolutivo. Una especie, según el **concepto fenotípico o morfológico**, es un grupo de individuos que comparte un conjunto concordante de estados de caracteres, y separado de otros grupos por discontinuidades en dichos estados de caracteres (Michener 1970; Sokal & Crovello 1970). La principal crítica a este concepto ha sido que puede estar sujeto a subjetividad a la hora de definir las diferencias fenotípicas. De acuerdo con el **concepto biológico** de especie introducido por Mayr (1942), la especie es un grupo de poblaciones naturales que no se cruzan y que están aisladas reproductivamente unas de otras. El principal problema de este concepto es que no puede ser aplicado a organismos de reproducción asexual. Según el **concepto filogenético** se considera que una especie es la agregación más pequeña de poblaciones (organismos sexuales) o linajes (organismos asexuales) diagnosticable por una combinación única de estados de caracteres (Nelson & Platnick 1981). Por otra parte, Donoghue (1985) y Mishler (1985) introducen el concepto de monofília en la definición de especie. Dichos autores consideran que una especie filogenética es el grupo monofilético más pequeño de organismos reconocidos por la posesión de estados de caracteres derivados. El **concepto evolutivo** de especie considera la especie como una entidad o conjunto de organismos que mantiene su identidad a través del tiempo y el espacio respecto de otros conjuntos de organismos, y que tiene sus propias tendencias históricas y evolutivas (Simpson 1951; Wiley 1978).

Los líquenes son el resultado de una asociación simbiótica estable entre un hongo, el micobionte, y una o más especies de algas verdes o cianobacterias, el fotobionte. En los estudios taxonómicos y en la delimitación de especies en líquenes se ha prestado más atención al micobionte que al fotobionte. El concepto más empleado para la delimitación de especies en los hongos liquenizados ha sido, hasta hace poco tiempo, el fenotípico. Los caracteres morfológicos empleados para dicha delimitación han sido: la morfología y coloración del talo; la presencia o ausencia de rizinas, de pseudocifelas, de pruína o de cilios; el tipo de reproducción (sexual o asexual); la posición de los ascomas o estructuras de reproducción asexual sobre el talo; la presencia de cristales y su morfología; el tamaño, forma y color de los conidios y de las ascósporas; así como los metabolitos secundarios (Ott & Lumbsch 2001; Lumbsch & Leavitt 2011). La mayoría de los caracteres morfológicos exhiben una gran variabilidad, lo que condujo a que los metabolitos secundarios adquirieran una gran importancia para distinguir especies. Culberson (1969) indicó que los extrolitos (metabolitos secundarios) eran mejores indicadores que los caracteres

morfológicos para identificar especies. Este punto de vista dio como resultado el distinguir especies que sólo discrepaban en su contenido en metabolitos secundarios. Más tarde, algunos autores criticaron esta postura (Hawksworth 1976; Lumbsch 1998), considerando que solo los quimiótipos que fuesen acompañados por diferencias en su distribución podían ser considerados como especies independientes (Hawsworth 1976), o que, además de las diferencias en su distribución, los quimiótipos debían ir acompañados de diferencias morfológicas para que pudiesen adquirir el nivel de especie (Lumbsch 1998).

Sobre la base del concepto fenotípico, el principal problema para la delimitación de especies en los hongos liquenizados es que presentan una organización muy simple, lo que hace que posean pocos caracteres morfológicos que puedan ser utilizados en taxonomía. Es imposible utilizar el concepto fenotípico cuando no está claro dentro de las especies qué caracteres son polimórficos y qué caracteres son monomórficos (Grube & Kroken 2000). Por otro lado, el concepto biológico de especie tampoco puede ser aplicado a los hongos liquenizados, puesto que no han podido ser cruzados en condiciones de laboratorio (Ahmadjan 1993).

Los primeros estudios en los que se aplicó el concepto filogenético de especie en líquenes utilizaron la región intragénica del DNA ribosómico nuclear (ITS rDNA) para reconocer la monofilia (DePriest & Been 1992; Niu & Wei 1993; Groner & LaGreca 1997; Crespo & Cubero 1998). Estos estudios empezaron a revelar que, en muchos casos, la interpretación de los caracteres fenotípicos había sido errónea. Se revisó la delimitación de especies en los llamados pares de especies (Poelt 1970), donde dos taxones que sólo se diferencian por el tipo de reproducción, sexual o asexual, eran considerados como especies independientes. En la mayor parte de los casos, los datos de las secuencias de DNA revelaron que los especímenes con diferente tipo de reproducción no constituían linajes monofiléticos independientes (Lohtander et al. 1998; Myllys et al. 2001; Articus et al. 2002; Buschbom & Mueller 2006; Crespo & Pérez-Ortega 2009). También ha sido revisada la interpretación del valor taxonómico de los metabolitos secundarios. En algunos casos, se ha encontrado que los diferentes quimiótipos no se correlacionaban con linajes monofiléticos (Buschbom & Mueller 2006; Nelsen & Gargas 2009), mientras que en otros casos sí que están correlacionados (LaGreca 1999; Tehler & Källersjö 2001; Lücking et al. 2008).

En la actualidad, el criterio más extendido para la delimitación de especies en los hongos liquenizados es el **reconocimiento filogenético de especies mediante concordancia genealógica** (Taylor et al. 2000). Este método requiere emplear más de un loci. La concordancia entre las topologías de los árboles resultantes de los diferentes genes indica que los polimorfismos se han fijado después de que se haya producido aislamiento genético. La transición entre la concordancia y la discordancia de genes corresponde al límite entre las especies; las incongruencias entre los diferentes genes indican recombinación intraespecífica.

Los genes más usados en los análisis filogenéticos de los hongos liquenizados son los genes ribosomales (nrSSU y nrLSU) y los espaciadores intergénicos (ITS rDNA). La variación de la región ITS rDNA se considera suficiente para estudios filogenéticos infragenéricos o incluso para estudios intraspecíficos (Bridge & Hawksworth 1998). Otros loci empleados en la delimitación de especies en hongos son el gen *rpb1*(Frøslev et al. 2005; Bischoff et al. 2006; Seymour et al. 2007; Wirtz et al. 2008), *rpb2* (Buschbom & Mueller 2006; Tehler & Irestedt 2007), la *β-tubulina* (Articus et al. 2002; Crespo et al. 2002; Myllys et al. 2003; Högnabba & Wedin 2003; Molina et al. 2004), los genes ribosomales mitocondriales mtSSU (Divakar et

al. 2005; Högnabba & Wedin 2003; Argüello et al. 2007) y mtLSU (Printzen 2002; Ott et al. 2004), el gen *ef1α* (Yarh et al. 2006; Jaklitsch et al. 2008) la región IGS rDNA (Lindblom & Ekman 2006; Wirtz et al. 2008) y el gen GAPDH (Myllys et al. 2003; Wedin et al. 2004).

EL GÉNERO *CLADONIA* HILL EX BROWNE 1756

El género de hongos liquenizados *Cladonia* está incluido en la familia Cladoniaceae, orden Lecanorales, Ascomycota (Miadlikowska et al. 2006; Lumbsch & Huhndorf 2011). Los fotobiontes son algas verdes del género *Asterochloris* (Tschermak-Woess 1989). El género *Cladonia* contiene más de 500 especies en todo el mundo y se caracteriza por la presencia de un talo compuesto, formado por un talo primario y un talo secundario (Ahti 2000). La mayoría son conspicuas y fácilmente reconocibles debido al color del talo y su forma característica. El talo primario puede ser crustáceo o foliáceo (FIG. 1), mientras que el talo secundario, llamado podecio, es fruticuloso (FIG. 2). Sobre la base de estudios ontogenéticos, Jahns & Beltman (1973) concluyen que los podecios son parte del "tejido generativo" (conjunto de hifas originadas de la unión del ascogonio y la tricogina y por tanto binucleadas), mientras que Hammer (1993, 1995) los considera como "tejido primario" del liquen (conjunto de hifas estrechamente empaquetadas en las cuales no ha ocurrido la plasmogamia y por tanto haploides). Los conidiomas en algunas especies se desarrollan sobre el talo primario, mientras que en otras lo hacen en el extremo de los podecios. Los ascomas son apotecios biatorinos; en la mayor parte de las especies se localizan en el ápice de los podecios, mientras que en raras ocasiones nacen directamente en las escuámulas del talo primario. Los ascos tienen un tolus de tipo *Porpidia* Körber (tipo de tolus en el cual sólo el tubo central es amiloide, mientras que la parte lateral no lo es) (Hafellner 1984) y contienen 8 ascósporas que son, en general, hialinas y simples. La mayor parte de las especies de *Cladonia* se reproducen principalmente de forma asexual, bien por medio de soredios o mediante fragmentación del talo, actuando los fragmentos como propágulos vegetativos. El género *Cladonia* tiene una gran variedad de metabolitos secundarios que, de acuerdo con Houvinen & Ahti (1982), son importantes en la sistemática del género. En concreto, la taxonomía del género se basa en los siguientes caracteres: 1) la morfología y anatomía del talo primario, 2) la morfología, ramificaciones, superficie y anatomía de los podecios, 3) los propágulos vegetativos, 4) los conidiomas y 5) los metabolitos secundarios.

La mayor parte de las especies son terrícolas, heliófilas y requieren gran cantidad de humedad para su desarrollo (Ahti 2000). Por ello, son hábitats muy propicios para el crecimiento de las especies de *Cladonia* los bordes de carreteras, desmontes, dunas y campos abandonados (Robinson 1959); también, son frecuentes en el suelo de los bosques de coníferas (Hammer 1995), siendo escasas en zonas muy secas y pastoreadas (Stenroos et al. 1992). Son más abundantes sobre sustratos ácidos que sobre calcáreos, aunque no faltan especies que se desarrollan sobre sustratos calcáreos (Burgaz & Ahti 2009). Algunas crecen sobre tocones y madera en descomposición.

Un gran número de especies de *Cladonia* tiene distribución cosmopolita, encontrándose en todos los continentes. Otras, sin ser cosmopolitas, tienen una amplia distribución, estando presentes en todo el hemisferio Norte o en todo el hemisferio Sur. Un alto número de especies de *Cladonia* tiene distribución bipolar, es decir, se distribuyen en la zona ártica y antártica. Otras especies tienen distribuciones disjuntas; por ejemplo, algunas especies se encuentran en las zonas

templadas de Norteamérica y Europa (Thomson 1968). Un pequeño número de especies tiene distribuciones restringidas, endémicas para determinadas regiones geográficas.

Las divisiones infragenéricas del género *Cladonia* son diferentes según los distintos autores (Tabla 1). Vainio (1897) incluyó dentro de *Cladonia* a los géneros actuales *Pycnothelia* y *Clathrina* como subgéneros, al igual que *Cladina.* Mattick (1940), continuó incluyendo a *Pycnothelia* y *Clathrina* dentro de *Cladonia* y consideró al grupo *Cladina* como una subsección dentro de la sección *Perviae.* Ahti (2000) considera a *Cladina* como un género independiente de *Cladonia* y divide *Cladonia* en siete secciones. Stenroos et al. (2002) realizaron el primer estudio filogenético de *Cladonia* basado en la región ITS rDNA y en el gen que codifica para la *β-tubulina*, incluyendo 165 taxones. Estos autores encontraron que las siete secciones definidas por Ahti (2000) eran polifiléticas y que *Cladina* formaba un grupo monofilético dentro de *Cladonia*; en consecuencia propusieron una clasificación provisional dividiendo el género en tres Subdivisiones y cuatro Supergrupos.

Tabla 1. Las diferentes clasificaciones infragenéricas del género *Cladonia*

Vainio 1897	**Mattick 1940**	**Ahti 2000**	**Stenroos et al. 2002**
Cladonia	*Cladonia*	*Cladina*	*Cladonia*
Subgénero *Cladina*	Subgénero *Clathrina*	*Cladonia*	Subdivisión I
Subgénero *Clathrina*	Subgénero *Pycnothelia*	Sección *Ascyphiferae*	Subdivisión II
Subgénero *Pycnothelia*	Subgénero *Eucladonia*	Sección *Cladonia*	Supergrupo *Cladonia*
Subgénero *Cenomyce*	Sección *Clausae*	Sección *Cocciferae*	Subdivisión III
Serie A. *Cocciferae*	Subsección *Cocciferae*	Sección *Helopodium*	Supergrupo *Perviae*
a Subglaucescentes	Subsección *Ochroleucae*	Sección *Perviae*	Supergrupo *Cocciferae*
b Stramineoflavidae	Subsección *Foliosae*	Sección *Strepsiles*	Grupo *Cocciferae*
Serie B. *Ochrophaeae*	Subsección *Podostelides*	Sección *Unciales*	Grupo *Miniatae*
α.Clathrinae	Subsección *Thallostelides*		Supergrupo *Crustaceae*
β Unciales	Sección *Perviae*		Grupo *Amaurocraeae*
γ Chasmiriae	Subsección *Chasmariae*		Grupo *Divaricatae*
δ Clausae	Subsección *Unciales*		Grupo *Unciales*
	Subsección *Cladinae*		Grupo *Cladinae*

CARACTERES MORFOLÓGICOS DEL GÉNERO *CLADONIA*

Morfología y anatomía del talo primario

Excepto en unas pocas especies cuyo talo primario es muy característico (p. ej., *Cladonia strepsilis* (Ach.) Grognot), en general es muy difícil identificar la especie sólo a partir del talo primario. Puede ser crustáceo o escuamuloso, persistente o evanescente. El talo primario crustáceo es característico de las especies del grupo *Cladinae* (Ahti 1961), aunque no es fácil de observar, pues desaparece una vez que se desarrollan los podecios. Otro grupo de especies con talo primario evanescente (en este caso escuamuloso) es el formado por *Cladonia furcata* (Huds.) Schrad., *C. subrangiformis* Sandst., *C. scabriuscula* (Delise) Nyl. y especies afines incluidas en la Sección *Ascyphiferae* por Ahti (2000) y, en la actualidad, incluidas en el Supergrupo *Cladonia* (Stenroos et al. 2002). Algunas especies tienen un talo primario muy desarrollado, siendo más conspicuo que los podecios, bien porque estos son de pequeño tamaño o bien porque los podecios se desarrollan en raras ocasiones. Se

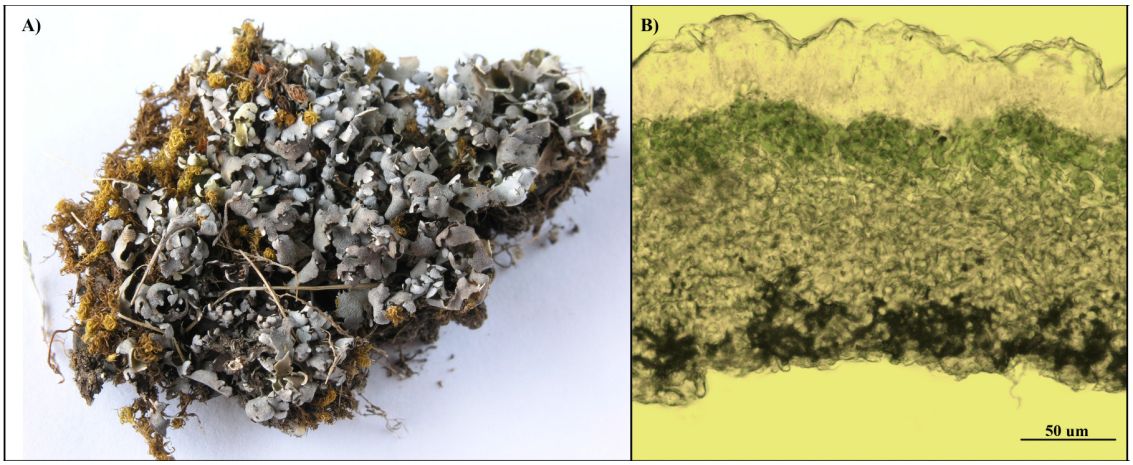

FIGURA 1. A) Talo primario escuamuloso dominante (*Cladonia iberica*) B) Corte transversal de una escuámula del talo primario de *Cladonia iberica*

consideró que la presencia de un talo primario dominante era importante en taxonomía y estas especies fueron agrupadas por Ahti (2000) en la sección *Helopodium*. Actualmente sabemos que este conjunto de especies no forma un grupo monofilético (Stenroos et al. 2002).

En los talos primarios escuamulosos (FIG. 1A), las escuámulas pueden ser enteras o estar divididas de forma variable hasta llegar a ser laciniadas; pueden estar imbricadas unas con otras o cada una separada de las otras; desarrollarse paralelas al sustrato o ser ascendentes; y, en ocasiones, pueden estar cuarteadas. Los márgenes con frecuencia están curvados hacia la cara superior. En cuanto a la coloración, la cara superior de las escuámulas puede ser, según las especies, parda, verde glauca, verde olivácea o gris. La cara inferior puede ser de color blanco, pardo, negro, amarillo, o con zonas anaranjadas. En ocasiones, de los márgenes de la cara inferior de las escuámulas pueden nacer cilios. Sin embargo, este carácter suele ser variable dentro de una misma especie, como en *C. foliacea* (Huds.) Willd o *C. firma* (Nyl.) Nyl. Otras especies, como *C. ceratophylla* (Sw.) Spreng., desarrollan rizinas (Ahti 2000).

Respecto a su anatomía, las escuámulas del talo primario están constituidas por un córtex prosoplectenquimático de grosor variable, la capa algal y la medula (FIG. 1B). En general carecen de córtex inferior, lo que origina que la cara inferior tenga un aspecto aracnoide. El grosor de las escuámulas, así como el de cada una de sus capas, no se ha utilizado mucho en taxonomía debido a que puede variar en función de las condiciones ambientales (Ahti 2000).

Los podecios: morfología, ramificaciones, superficie y anatomía

Los podecios, o talo secundario, son fruticulosos, nacen del talo primario y, en general, perpendiculares al sustrato. La taxonomía clásica del género está basada en las características morfológicas de los podecios. En la mayor parte de las especies están huecos y sólo en unas pocas especies son macizos (*C. solida* Vain. y *C. stereoclada* Abbayes). De acuerdo con la morfología del ápice, los podecios se clasifican en subulados (FIG. 2A), aquellos que presentan ápices agudos, o escifosos, aquellos que se ensanchan formando una estructura que recuerda a una copa (FIG. 2B-D). Los dos tipos de podecios pueden ser simples o estar ramificados de forma variable, aunque en los podecios escifosos las ramificaciones pueden nacer también del centro del escifo, como en el grupo de *C. verticillata* (Hoffm.) Schaer. (Ahti

2007), o del borde del mismo como en el grupo de *C. gracilis* (Ahti 1980); la parte interna de los escifos puede ser plana, cóncava, estar cerrada o perforada. Los podecios subulados pueden ser simples o estar ramificados de forma variable. El número, el tipo y el patrón de ramificación son muy importantes para separar las especies. Así, en la mayoría de las claves de identificación al uso es uno de los primeros pasos utilizados (James 2009; Ahti 2000; Burgaz & Ahti 2009). Las ramificaciones pueden ser dicótomas, tricótomas, tetracótomas hasta polítomas; de igual tamaño, y se dice entonces que son isótomas (FIG. 3A), o bien las diferentes ramas pueden tener diferente longitud, calificándose entonces de anisótomas (FIG. 3B). Otro carácter importante para identificar las especies se encuentra en las axilas de las ramificaciones, que pueden ser cerradas o perforadas.

La superficie de los podecios proporciona un carácter importante para la delimitación de especies, ya que puede estar corticada (FIG. 4A) o carecer de córtex (FIG. 4B). Los podecios que carecen de córtex tienen un aspecto afieltrado (FIG. 4B), característico de las especies del grupo *Cladinae.* La capa algal puede ser continua o discontinua y en este último caso la superficie del podecio tiene aspecto areolado (FIG. 4C). Los podecios pueden estar corticados en toda su superficie o sólo en una zona, como por ejemplo en *C. cornuta* (L.) Hoffm., cuyos podecios están corticados solo en los dos tercios inferiores. Muchas especies tienen la superficie de los podecios recubiertas por soredios (FIG. 4D), como por ejemplo *C. fimbriata* (L.) Fr. o *C. subulata* (L.) F.H. Wigg.

Otro carácter importante a nivel específico es la presencia de escuámulas sobre la superficie de los podecios (FIG. 4E) y su localización (restringida a la base o distribuidas en toda la longitud del podecio).

Respecto a la anatomía, en los podecios se pueden distinguir diferentes capas desde el exterior al interior: el córtex, la capa algal, la medula y el estereoma. El córtex es un tejido prosoplectenquimático que, como se ha comentado en el apartado anterior, puede faltar en muchas especies. La medula es un tejido aracnoide; en muchas especies de *Cladonia* la capa algal de los podecios no está bien delimitada, es discontinua y se considera parte de la medula. La capa más interna es el estereoma, una capa prosoplectenquimática, cartilaginosa, que está constituida exclusivamente por hifas del micobionte. Las diferencias en el grosor total de la pared del podecio, o de las diferentes capas del mismo, se han utilizado para distinguir especies estrechamente relacionadas (Ahti 1980; Burgaz & Martínez 2008). La superficie del estereoma puede ser lisa o estriada, siendo este carácter usado para distinguir a *C. furcata* de sus taxones afines (Ahti 2000).

Conidiomas

Los conidiomas en *Cladonia* son picnidios. La morfología de los picnidios varía durante su desarrollo y, además, algunas especies pueden tener picnidios con diferentes formas en su estado maduro. A pesar de esta variabilidad, Stenroos (1994, 1998) encontró que los picnidios contenían caracteres útiles para distinguir especies. Los picnidios pueden aparecer sobre las escuámulas del talo primario o sobre los podecios; su posición fue utilizada por Jahns & Beltman (1973) para definir diferentes tipos de ontogenia de los discos himeniales. Los picnidios pueden ser piriformes, cilíndricos, elongados, globosos, estipitados, o pueden estar constreñidos en la base; en la mayor parte de las especies son de color negro o pardo oscuro, aunque algunas especies los tienen de color rojo oscuro (Stenroos 1994). Los

conidios o picnidiosporas son hialinas y falciformes y, de acuerdo con Stenroos (1998), con frecuencia carecen de utilidad taxonómica.

Ascomas y ascósporas

A diferencia de lo que ocurre en otros géneros de líquenes, los apotecios y las ascósporas tienen poca importancia en la delimitación de especies en *Cladonia*, debido a que muchas especies sólo en raras ocasiones desarrollan apotecios, mientras que las ascósporas son uniformes en todas las especies (Ahti 2000). El color de los apotecios (pardo, rojo y rosa) ha sido uno de los caracteres utilizados para dividir el género en grupos infragenéricos. En la actualidad se ha demostrado que el color rojo de los apotecios es un estado sinapomórfico que caracteriza al Supergrupo *Cocciferae* (Stenroos et al. 2002).

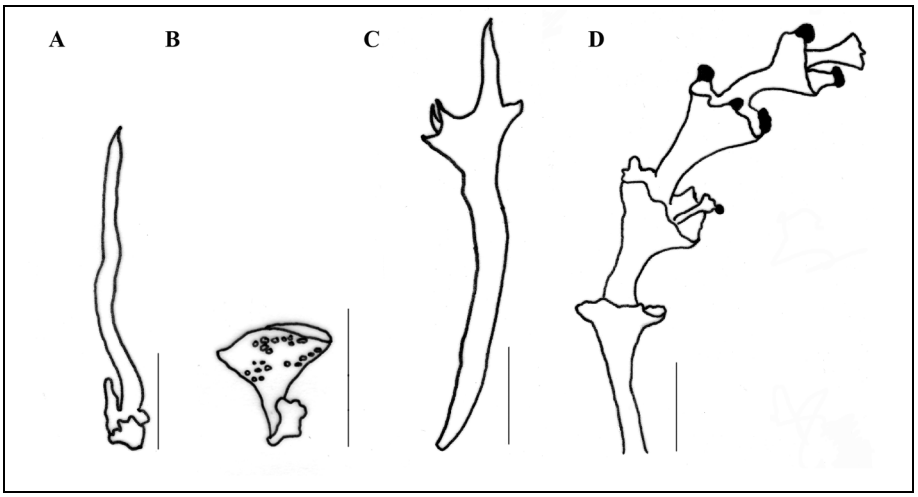

FIGURA 2. Diferentes tipos de podecios. A) Podecio subulado no ramificado B) Podecio escifoso simple C) Podecio escifoso con ramificación lateral D) Podecio escifoso con ramificaciones centrales.

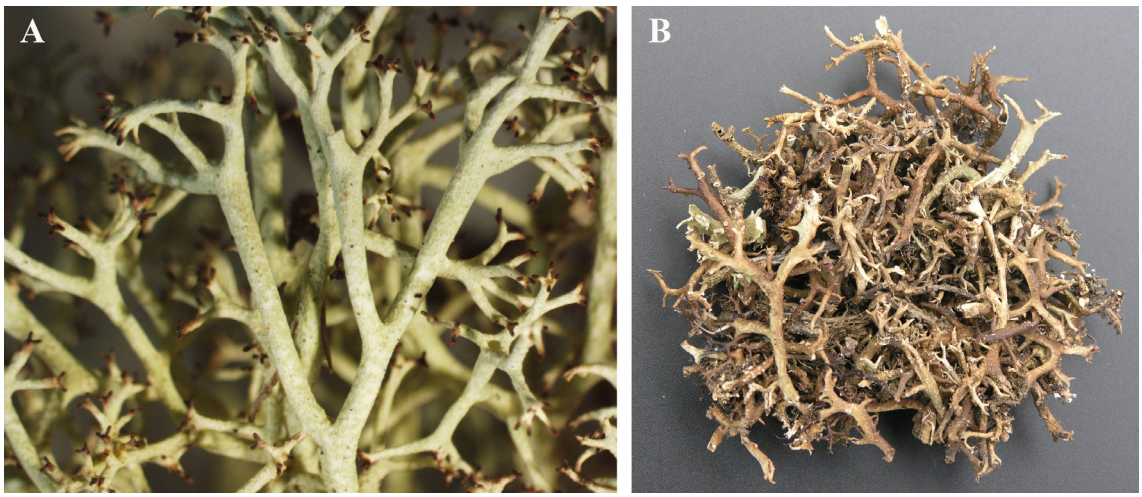

FIGURA 3. A) Podecio con ramificaciones isotómicas B) Podecio con ramificaciones anisótomas.

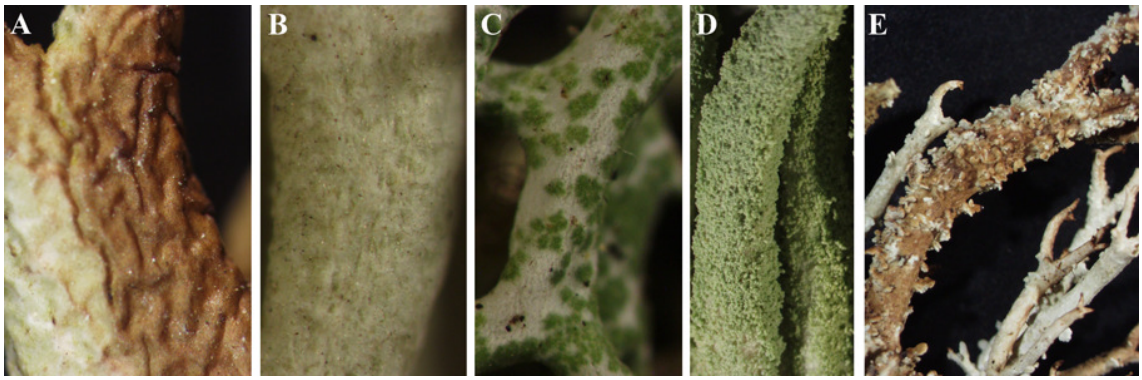

FIGURA 4. Superficie de los podecios. A) Superficie corticada B) Superficie sin córtex C) Superficie areolada D) Superficie sorediada F) Podecio provisto de escuámulas.

Propágulos vegetativos

Los soredios son los propágulos vegetativos más comunes en las especies de *Cladonia*, y tanto su localización como su tamaño se han utilizado en la delimitación de especies. Stenroos (1989) clasificó los soredios en farináceos, cuando tienen un diámetro inferior a 30 μm, y granulares, cuando tienen un diámetro superior a 30 μm. En la mayoría de las especies que desarrollan soredios, estos se disponen en soralios difusos, es decir, sin una forma definida, cubriendo la superficie de los podecios, mientras que en otras especies, como en *C. ochrochlora* Flörke, los soredios se pueden agrupar en soralios ovales (Ahti & Hammer 2002). Algunas especies también desarrollan soredios en la cara inferior de las escuámulas del talo primario.

Muchas especies desarrollan gránulos corticados (> 100 μm) que actúan como propágulos vegetativos. La morfología de estos gránulos (peltados, bullados, planos, etc.) permite distinguir especies, como las del grupo de *C. pyxidata* (Aptroot et al. 2001). Por otro lado, los isidios son muy raros en *Cladonia,* aunque algunas especies los desarrollan, como *C. caribea* S. Stenroos (Ahti 2000).

METABOLITOS SECUNDARIOS

Los extrolitos o metabolitos secundarios en *Cladonia* se han usado para caracterizar entidades taxonómicas de distinto rango, en particular, para identificar especies, aunque de los cerca de 60 compuestos químicos identificados en *Cladonia*, sólo 30 de ellos tienen importancia taxonómica (Ahti 2000), siendo el resto compuestos satélites que acompañan a los otros. Por ejemplo, el ácido fumarprotocetrárico suele ir acompañado del ácido protocetrárico. Muchas especies tienen varios quimiótipos, por lo que su estatus taxonómico ha sido discutido. Los compuestos más comunes en el género *Cladonia* son los siguientes: las depsidonas, como los ácidos fumarprotocetrárico, protocetrárico, confumarprotocetrárico, psorómico, estíctico, norestíctico homosekikaico, sekikaico, meroclorofeico o perlatólico; los dépsidos, entre los cuales los más comunes en *Cladonia* son los ácidos barbático, escuamático, tamnólico, grayánico y la atranorina; los dibenzofuranos, entre los cuales el ácido didímico es el más común; el ácido úsnico comun en varios grupos de especies (por ejemplo, el grupo de *C. uncialis* o el grupo de *C. coccifera*); los terpenos, el más frecuente de los cuales es la zeorina; los ácidos grasos, representados principalmente por los ácidos rangifórmico, bourgeánico y protoliquesterínico.

JUSTIFICACIÓN DEL TRABAJO: PROBLEMAS PARA LA DELIMITACIÓN DE ESPECIES EN EL GÉNERO *CLADONIA*.

Como ya se ha indicado, las especies del género *Cladonia* son conspicuas, tienen distribución cosmopolita y ocupan un gran número de ambientes. Por esta razón es necesario resolver los múltiples problemas que los naturalistas e investigadores encuentran en la determinación de sus especies. La dificultad de la delimitación de especies en *Cladonia* mediante caracteres morfológicos radica en la alta variabilidad de la mayoría de la especies, es decir, en el hecho de que, en general, son escasos los caracteres específicos que se mantienen constantes en los distintos especímenes. Para la identificación de especies de *Cladonia,* en la mayor parte de los casos se necesita estudiar varios podecios por espécimen para poder encontrar todos los caracteres específicos. Varios autores han estudiado en detalle la variación morfológica intraspecífica de *Cladonia* (Vainio 1897; Thomson 1968; Hammer 1996; Ahti 2000). Muchas especies, como *C. gracilis* (L.) Willd., tienen la capacidad de producir tanto podecios escifosos como podecios subulados; incluso se pueden encontrar podecios en los que unas ramas están provistas de escifos y otras son subuladas. En algunas especies, como *C. subcervicornis,* se ha encontrado que el hecho de que los podecios presenten escifos, o no, depende de cambios tempranos en el desarrollo (Hammer 1998). El número de ramificaciones en muchas especies también es variable; es frecuente que ciertos especímenes de algunas especies presenten un aumento del número de ramificaciones respecto a lo que es común en la especie. También se puede dar el caso contrario, una reducción del número de ramificaciones. Por ello, en la descripción de las especies del grupo *Cladinae* es frecuente encontrar rasgos tales como "ramificaciones tricótomas o tetracótomas, algunas veces dicótomas". Otro carácter que en la mayor parte de las especies parece ser inconstante es la producción de escuámulas. Muchas especies que de forma normal desarrollan podecios desprovistos de escuámulas, bajo ciertas condiciones (en la mayor parte de los casos no determinadas) producen podecios provistos de escuámulas (p. ej., *C. jaliscana* Ahti & Guzmán-Dávalos). También hay especies que producen podecios sin soredios, aunque en ciertas circunstancias presentan podecios sorediados (p. ej., *C. rangiformis* Hoffm.).

La gran variabilidad morfológica que existe dentro de las especies llevó a que se describieran numerosos taxones infraspecíficos (Vainio 1887, 1894, 1897; Sandstede 1931; Evans 1944). En la actualidad, muchos de estos taxones han sido sinonimizados, ya que se considera que la variación morfológica es consecuencia de la acción de los factores ambientales sobre el talo. Sin embargo, en otros muchos casos ha sido difícil determinar de una forma clara qué modificaciones morfológicas son debidas a la plasticidad fenotípica de las especies y cuáles están correlacionadas con la variación genética. De modo que el rango específico de muchos taxones sigue siendo motivo de debate y numerosos grupos y complejos de especies necesitan una revisión taxonómica.

Los primeros estudios de delimitación de especies usando marcadores moleculares fueron realizados en el complejo de *Cladonia chlorophaea* (Flörke ex Sommerf) Spreng. (DePriest 1993a, 1993b, 1994), en los cuales no se encontró que existiese una correlación entre las diferentes especies y la variación del DNA ribosómico nuclear. Después, Myllys et al. (2003) estudiaron si *C. arbuscula* (Wallr.) Flotow y *C. mitis* Sandst. eran monofiléticas empleando para ello cuatro loci (ITS rDNA, SSU rDNA, *β-tubulina* y GAPDH). Los autores encontraron que *C. mitis* era genéticamente distinta de *C. arbuscula*; además, *C. arbuscula* resulto ser parafilética.

Al igual que en otros grupos de hongos liquenizados (Lothander et al. 2001; Tehler & Källersjö 2001; Högnabba & Wedin 2003; Ohmura 2009; Argüello et al. 2007) el análisis de los datos moleculares ayudará a determinar la relevancia taxonómica de los diferentes caracteres morfológicos y a evaluar la utilidad de los metabolitos secundarios en la delimitación de especies dentro del género *Cladonia.*

OBJETIVOS

OBJETIVOS

El objetivo general de esta memoria doctoral es estudiar la delimitación de algunas especies conflictivas en el género *Cladonia*, utilizando como criterio el reconocimiento filogenético de especies mediante concordancia genealógica.

Para cumplir este objetivo general se seleccionaron grupos de especies de *Cladonia,* frecuentes en la región Mediterránea, sobre los que diferentes autores discrepan respecto a los límites entre las especies y en cuanto a cuáles son los caracteres que tienen valor taxonómico interespecífico.

Para alcanzar el objetivo general se plantean los siguientes objetivos específicos:

1.- Determinar el valor de los caracteres morfológicos para la delimitación de especies en los grupos seleccionados (ARTÍCULOS I-VIII).
2.- Determinar el valor de los metabolitos secundarios para la delimitación de especies en los grupos seleccionados (ARTÍCULOS I-VIII).
3.- Determinar si, en cada grupo de especies, los taxones constituyen linajes independientes (ARTÍCULOS I-VIII).
4.- Determinar qué regiones del DNA pueden utilizarse para la identificación de especímenes de *Cladonia,* es decir, ser aptas para "DNA *barcoding"* (ARTÍCULO IX).

HIPÓTESIS

Los caracteres morfológicos cuantitativos tienen una utilidad limitada para diferenciar especies estrechamente relacionadas del género *Cladonia.*

La producción de diferentes metabolitos secundarios no siempre refleja la existencia de diferentes linajes evolutivos, sino que son las diferentes rutas metabólicas por las que se sintetizan los metabolitos secundarios las que tienen una mayor utilidad para diferenciar especies estrechamente relacionadas del género *Cladonia.*

MATERIAL Y MÉTODOS

MATERIAL Y MÉTODOS

MATERIAL ESTUDIADO

Para realizar este trabajo se han estudiado más de 3400 colecciones (ANEXO 1) procedentes de los herbarios B, BG, BRA, CANB, F, FH, FR, GAD, H, ICEL, L, MACB, MA-Lichen, S y UPS. (Index Herbariorum). Los grupos de estudio seleccionados, frecuentes en la región Mediterránea, son los siguientes:

1.- *Cladonia convoluta* (Lam.) Anders y *C. foliacea* (Huds.) Willd (681 especimenes estudiados).
2.- *Cladonia iberica* Burgaz & Ahti y *C. subturgida* Sampaio (188 especímenes estudiados).
3.- *Cladonia rei* Schaer. y *C. subulata* (L.) F.H. Wigg (301 especímenes estudiados).
4.- Grupo de *Cladonia cariosa* que incluye los taxones *C. acuminata* (Ach.) Norrl., *C. cariosa* (Ach.) Spreng. y *C. symphycarpa* (Flörke) Fr. (323 especímenes estudiados).
5.- Grupo de *Cladonia gracilis* que incluye los taxones *C. coniocraea* (Flörke) Spreng., *C. cornuta* (L.) Hoffm., *C. ecmocyna* Leight., *C. gracilis* (L.) Willd., *C. macroceras* (Delise) Hav. y *C. ochrochlora* Flörke (770 especímenes estudiados).
6.- *Cladonia conista* (Nyl.) Robbins y *C. humilis* (With.) J. R. Laundon (296 especímenes estudiados).
7.- Grupo de *Cladonia humilis* que incluye los taxones *C. conista, C. cyathomorpha* Stirt. ex Walt. Watson, *C. hammeri* Ahti, *C. humilis, C. kurokawae* Ahti & Stenroos, *C. nashii* Ahti, *C. pulvinella* Hammer, *C. subconistea* Asahina (79 especímenes estudiados).
8.- *Cladonia furcata* (Huds.) Schrad. y *C. subrangiformis* Sandst. (805 especímenes estudiados).

La delimitación de cada uno de los grupos se basó en el estudio filogenético del género *Cladonia* de Stenroos et al. (2002). A la hora de seleccionar las muestras para el aislamiento del DNA y los posteriores análisis moleculares se intentó que estuviera representado todo el rango morfológico, químico y geográfico de cada uno de los taxones. Siempre que fue posible, se seleccionó un mínimo de 10 muestras por taxón.

ANÁLISIS MORFOLÓGICOS

Todos los especímenes se estudiaron bajo la lupa binocular para revisar las identificaciones y seleccionar los especímenes para los estudios moleculares. En todos los especímenes seleccionados se realizó un estudio morfológico, para lo cual se midieron la longitud y anchura de las escuámulas, la longitud de las incisiones y la longitud de los podecios. Por lo que respecta a las escuámulas, cuando eran enteras, la anchura se midió a 2 mm del ápice; cuando eran divididas, a 2 mm de la incisión más profunda. A este nivel se hicieron cortes transversales para el estudio microscópico y se midió el grosor del talo, y de cada una de las capas que lo componen. Se tomaron estas medidas en tres escuámulas por talo, excepto cuando el material era escaso, en cuyo caso se tomaron medidas de una única escuámula. También, siempre que fue posible, se midió la longitud de las picnidiósporas (10 por muestra) y la longitud y anchura de las ascósporas (10 por muestra). Para medir el

grosor de cada una de las capas, se realizaron secciones a mano alzada en la base de los podecios y se tiñeron con azul de factofenol. Todas las medidas microscópicas se efectuaron a 400 aumentos.

Para cada grupo de especies se estudiaron los caracteres taxonómicos propuestos por los distintos autores para distinguir las especies (ver cada uno de los capítulos correspondientes). En el análisis morfológico del grupo de *C. subulata* y *C. rei* (ARTÍCULO III) y el grupo de *C. cariosa* (ARTÍCULO IV) se realizaron cortes semifinos (de unos 20 μm de grosor) mediante el micrótomo de congelación Micron-ACP. Los cortes se tiñeron con azul de lactofenol (Panrea), se observaron con el microscopio Olympus CX41 y se fotografiaron con la cámara MicroPublisher 5.0 (Qimaging). Las imágenes se procesaron con el programa AUTO-MONTAJE (Synoptics), que permite, a partir de una secuencia de imágenes con distintas zonas en foco, generar una imagen-montaje con todos los puntos en foco, de acuerdo con el método descrito en Diéguez-Uribeondo et al. (2003). También, en los estudios morfológicos de los ARTÍCULOS III y IV, se analizaron los podecios y las escuámulas mediante el microscopio electrónico de barrido (SEM). Se hicieron secciones longitudinales de los podecios para observar la estructura del estereoma (ARTÍCULO III); dichas secciones se colocaron sobre los portaobjetos de microscopía electrónica. Para la observación de la estructura del córtex (ARTÍCULO IV) se depositaron fragmentos de las escuámulas del talo primario sobre los portaobjetos de microscopía electrónica. La metalización se realizó mediante un baño de oro con el metalizador Balzer SCD 004. Las observaciones se realizaron en el microscopio electrónico Hitachi S-3000N del Real Jardín Botánico, CSIC.

CROMATOGRAFÍA EN CAPA FINA (TLC)

El análisis de los metabolitos secundarios presentes en las especies estudiadas se realizó mediante cromatografía en capa fina (TLC), de acuerdo con el método estandarizado de White & James (1985). La extracción de sustancias liquénicas de los talos se realizó sumergiendo fragmentos de los talos en acetona durante 10 minutos. Con un capilar de vidrio se tomó el extracto de acetona y se aplicó sobre la placa cromatográfica de Silicagel 60 F_{254} (MERK, Alemania). La separación de las sustancias liquénicas se realizó en dos fases móviles distintas, A (180 ml de tolueno, 60 ml de dioxano y 8 ml de ácido acético) y B (130 ml de hexano, 100 ml de éter etílico y 20 ml de ácido fórmico). Para revelar las placas se pulverizaron con ácido sulfúrico al 10%. Posteriormente, las placas fueron introducidas en una estufa a 100-110 ºC durante 10 minutos. La atranorina (con una clase de Rf = 7) y el ácido norestíctico (con una clase de Rf = 4) fueron utilizados como sustancias patrones para identificar el resto de sustancias mediante las tablas de White & James (1985).

EXTRACCIÓN DE DNA

De cada una de las muestras seleccionadas para los análisis moleculares se utilizó un único podecio para la extracción de ADN (entre 5 y 50 mg de peso seco aproximadamente), excepto para aquellos taxones en los cuales el talo primario es predominante y sólo en raras ocasiones desarrollan podecios. Consideramos importante utilizar un único podecio para la extracción, ya que los estudios de DePriest (1993a) encontraron que un simple individuo ("mat") contenía más de un genotipo. Previamente a la extracción de ADN, las muestras se lavaron con acetona. Se realizó un primer lavado con 100 μl de acetona durante 30 minutos; la acetona del

primer lavado se utilizó para confirmar la presencia de metabolitos secundarios de la muestra mediante TLC. A continuación, se realizó un segundo lavado con 500 µl de acetona durante dos horas con objeto de eliminar la mayor cantidad posible de metabolitos secundarios que pudieran dificultar la posterior amplificación del ADN.

El aislamiento de ADN se realizó mediante el kit E.Z.N.A Fungi DNA Miniprep Kit (Omega Biotech) (ARTÍCULO I y II) o con el kit DNeasy Plant (QIAGEN) (ARTÍCULO II, III-VIII). En ambos casos se siguieron las instrucciones del fabricante, con una única modificación: la incubación con el primer tampón se realizó a 60 ºC durante 12 horas (el protocolo indica mantener las muestras 10 minutos a 65 ºC) según Martín et al. (2000). La concentración del ADN extraído se cuantificó con el espectrofotómetro RNA/DNA Calculator GeneQuant II (Pharmacia Biotech), o bien mediante una electroforesis en gel de agarosa al 2%.

SELECCIÓN DE MARCADORES GENÉTICOS

Se decidió emplear 3 o 4 loci por grupo para resolver los problemas taxonómicos planteados en este estudio. Para la elección de estos marcadores se consultó la bibliografía y se hicieron pruebas para estudiar la facilidad de amplificación y la variabilidad de cada uno de los loci empleados por otros autores para la delimitación de especies en hongos. Las pruebas se realizaron con 8 muestras de *C. convoluta* y *C. foliacea* (incluyendo toda la variación morfológica del complejo), 1 muestra de *C. firma* y otra de *C. cervicornis* (Ach.) Flot.. Se decidió utilizar la región **ITS rDNA** porque ha sido el marcador más empleado para establecer las relaciones filogenéticas en los hongos liquenizados; además, el EMBL/GenBank almacena un gran número de secuencias de esta región que podían ser usadas en los análisis filogenéticos en caso necesario. La Tabla 2 resume las pruebas realizadas con los diferentes marcadores genéticos y los cebadores utilizados están indicados en la Tabla 3. Para la región **nrLSU** se obtuvo un éxito de amplificación cercano al 100%, pero la mayor parte de los cromatogramas presentaron dobles picos, convirtiendo las secuencias en ilegibles. La región **nrSSU** también se amplificó con gran éxito (aunque menor que el de la región nrLSU); la mayor parte de las secuencias tenían buena calidad pero se encontraron hasta 4 intrones, no presentes en todas las muestras. Aún así, descartamos esta región por el bajo número de posiciones variables que presentaba. Se intentó amplificar dos fragmentos diferentes del gen ***rpb2***, la región comprendida entre los dominios 5 y 7 y la región comprendida entre los dominios 7 y 11 El éxito de amplificación para ambas regiones fue bajo, pero la región comprendida entre los dominios 5 y 7 fue más fácil de amplificar (sólo se obtuvo una secuencia entre los dominios 7 y 11) y contenía un gran número de posiciones informativas. Por tanto, se seleccionó la región comprendida entre los dominios 5 y 7 del gen *rpb2* para los análisis filogenéticos del presente estudio. Las pruebas realizadas con gen ***rpb1*** tuvieron un éxito bajo de amplificación y se decidió descartar esta región. Se realizaron varias pruebas para amplificar el gen de la ***β-tubulina,*** pero no se consiguió amplificación (Tabla 3). Además, se decidió descartar este marcador debido a los problemas asociados de paralogía que han sido descritos (Begerow & Oberwinkler 2004). El gen ***cox1*** se amplificó con gran facilidad y además se encontró un gran número de posiciones variables. Por tanto, se escogió este marcador para los análisis filogenéticos del ARTÍCULO I. En las pruebas realizadas de la región **mtSSU** se obtuvo un éxito de amplificación cercano al 100% (Tabla 2). Sin embargo el número de posiciones variables fue muy bajo, por lo que este marcador fue desechado. Para probar el gen ***ef1α*** se utilizaron 10 muestras del grupo de *C. cariosa*.

Los resultados en cuanto a éxito de amplificación y variabilidad fueron buenos, por lo que se seleccionó este marcador para los análisis filogenéticos de los ARTÍCULOS III, IV, V, VI y VII. En la presente tesis se intentó amplificar la región **IGS rDNA** con dos pares de cebadores, CNL12/5SAr y IGSf/IGSr (utilizando muestras de *C. furcata*, *C. subrangiformis* y *C. rangiformis*). Con el primer par se obtuvieron múltiples bandas poco intensas. Posteriormente se hicieron pruebas con el segundo par de cebadores IGSf/IGSr, obteniéndose una única banda muy intensa. El porcentaje de sitios variables era semejante al de ITS rDNA o mayor, y se decidió utilizar este marcador para los ARTÍCULOS V, VII y VIII. Las pruebas realizadas con el gen **GAPDH** dieron como resultado un bajo éxito de amplificación, obteniéndose múltiples bandas poco intensas. Cuando se modificó el programa de amplificación subiendo la temperatura de hibridación no se obtuvo amplificación, por lo que se descartó este marcador. La región **mtLSU** se consideró como un posible candidato sobre la base de los resultados obtenidos por Printzen (2002) y Ott et al. (2004). Se decidió utilizar este marcador para estudiar la independencia de *C. iberica* y *C. subturgida* (ARTÍCULO II).

Tabla 2. Éxito, calidad de las secuencias y posiciones variables de las pruebas realizadas para cada uno de los marcadores genéticos. – No amplificación, + < 30% de éxito de amplificación, ++ 30-70% de éxito de amplificación, +++ 70-90% de éxito de amplificación, ++++ > 95% de éxito de amplificación. (1) amplificación con los cebadores CNL12/5Sar, (2) amplificación con los cebadores IGSf/IGSr.

Marcador	Éxito de PCR	Calidad de las secuencias	Sitios del alineamiento	Sitios variables
nrSSU	+++	Buena	1725	5
nrLSU	++++	Picos dobles	1490	18
rpb2 (5-7)	+	Buena	998	67
rpb2 (7-11)	+	Buena	–	–
rpb1	+	Buena	766	25
mtSSU	++++	Buena	897	17
cox1	++++	Buena	683	49
β-tubulina	–	–	–	–
ef1α	++++	Buena	622	33
IGS rDNA[1]	+	–	–	–
IGS rDNA[2]	++++	Buena	367	56
GADPH	+	–	–	–

AMPLIFICACIÓN Y PURIFICACIÓN DEL ADN

La mayor parte de las PCR se realizaron mediante Ready-to-Go PCR Beads (GE Healthcare Life Science, UK), en las condiciones, y con los programas de PCR, que se indican en cada uno de los capítulos. Sólo se utilizó la polimerasa Biotaq (Ecogen) para la amplificación de la región IGS rDNA en el ARTÍCULO VII. Los cebadores que se utilizaron en los artículos de esta tesis se indican en la Tabla 3.

En algunas muestras, debido a que el material era antiguo o a que los cebadores eran inespecíficos, la amplificación del ADN falló mediante PCR convencional. En estas muestras se procedió a realizar **PCR por partes** o **nested-PCR** (PCR anidada). La técnica de **PCR por partes** se utilizó para amplificar la región ITS rDNA (ARTÍCULO VIII). Esta técnica consiste en realizar dos PCRs amplificando fragmentos más cortos. Se utilizó el par de cebadores ITS1F/ITS2 para

amplificar la primera parte, y los pares ITS3/ITS4 o ITS3/LR15 para amplificar el segundo fragmento. La amplificación de cada una de las partes con frecuencia produjo varias bandas que fueron cortadas, purificadas y secuenciadas. La **nested-PCR** se utilizó para amplificar el gen *rpb2* (ARTÍCULOS II-VIII). Esta técnica consiste en realizar dos PCRs consecutivas. En la segunda PCR, el ADN molde es el producto de amplificación de la primera PCR. Los cebadores que se utilizan en la segunda PCR hibridan en posiciones más internas que los cebadores de la primera PCR. Los cebadores utilizados en la primera PCR para amplificar el gen *rpb2* fueron RPB2-5F/RPB2-7R, y los utilizados en la segunda PCR fueron RPB2dRaq/RPB2rRaq.

Para comprobar que el ADN se había amplificado, y la calidad del mismo, se realizaron electroforesis en geles de agarosa al 2% (Agarosa D-1 low EEO, laboratorios Pronadisa) con tampón TAE 1x. El gel contenía SYBRgreen (Invitrogen) (1 l/10ml). En los pocillos se aplicaron 5 l del producto de amplificación. La electroforesis se desarrolló durante 30 minutos a 100 V para un tamaño de gel de 7x10 cm. El marcador de peso molecular utilizado fue 1Kb Plus ADN Ladder (Invitrogen).

En los casos en los que se visualizó más de una banda o una única banda con rastro, los amplímeros se purificaron mediante el Kit QIAquick (Qiagen, Valencia. California, USA). Para ello, en primer lugar, tras el gel de comprobación se preparó un nuevo gel (7x10 cm) con el mismo tipo de agarosa, en el que los pocillos se cargaron con el resto del producto amplificado (unos 20 l). La electroforesis se realizó durante 45 minutos a 120 V, se cortó la banda de interés y, a continuación, se realizó la purificación siguiendo las instrucciones del fabricante. El ADN se resuspendió en 40 l del tampón de elución que contiene el kit. Para comprobar que la concentración del producto purificado (> 20 ng/ml) era suficiente para la secuenciación se hizo una electroforesis en gel de agarosa al 2% (Agarosa D-1 low EEO), con 5 l del producto purificado. Cuando el producto de la PCR fue una única banda, intensa sin rastro, la purificación se realizó mediante la enzima ExoSap-IT (USB Corporation, OH, USA). A cada producto de la PCR se le añadió 8 l de enzima y se siguieron las instrucciones del fabricante.

Las reacciones de secuenciación se realizaron en el servicio de secuenciación del CIB (CSIC, Madrid) o en Macrogen (Corea del Sur), utilizando 5 l del producto purificado y los mismos cebadores que se utilizaron en la PCR.

DISEÑO DE CEBADORES

Se diseñaron dos pares de cebadores, uno para el gen *rpb2* y otro para la región ITS rDNA. Para diseñar un par de cebadores más específicos para el locus *rpb2* (ARTÍCULO III) se utilizaron las secuencias de *C. foliacea*, *C. cervicornis*, *C. firma* y *C. pulvinata* (Sandst.) Van Herk & Aptroot (ARTÍCULO I) y además se descargaron del GenBank las secuencias de *Cladonia subtenuis* (Abbayes) Mattick (DQ522282-DQ522289). Para diseñar un par de cebadores para la región ITS rDNA se utilizó el alineamiento de la región ITS rDNA del ARTÍCULO I. Todas las secuencias fueron alineadas mediante SE-AL v2.0a11 (Rambaut 2002).

Para diseñar los cebadores se tuvieron en cuenta las siguientes condiciones: que el porcentaje de GC fuese cercano al 50%; que la longitud del cebador fuese de 20 o 21 bp y que no hibridasen entre ellos. Como ayuda para elegir la región idónea se utilizó el pograma Primer3 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). La síntesis de los cebadores fue encargada a DNA technology A/S (Dinamarca).

Tabla 3. Lista de cebadores utilizados durante el desarrollo de este trabajo. DR = dirección: D = cebador directo, R = cebador reverso.

Locus	Cebador	Secuencia (5'-3')	DR	Referencia	Artículo
ITS nrDNA	ITS1F	cttggtcatttagaggaagtaa	D	Gardes & Bruns 1993	I-VIII
	ITS4	tcctccgcttattgatatgc	R	White et al. 1990	I-VIII
	ITS1	tccgtaggtgaacctgcgg	D	White et al. 1990	VIII
	ITS2	gctgcgttcttcatcgatgc	R	White et al. 1990	VIII
	ITS3	gcatcgatgaagaacgcagc	D	White et al. 1990	VIII
	1780-5F	ctgccggaaggatcattaatgag	D	Piercey-Normore & DePriest 2001	III, VI, VII
	LSU0012-3'	agttcagcgggtatccct	R	Piercey-Normore & DePriest 2001	III, VI, VII
nrSSU	NS17	catgtctaagttaagcaa	D	Gargas & Taylor 1992	Pruebas
	NSSU1088	tgatttctcgtaaggtgccg	R	Kauff & Lutzoni 2002	Pruebas
nrLSU	LROR	acccgctgaacttaagc	D	Rehner & Samuels 1994	Pruebas
	LR7	tactaccaccaagatct	R	Vilgalys & Hester 1990	Pruebas
nrLSU	LR15	taaattacaactcggac	R		
rpb2	RPB2-5F	gaygayngwgatcayttygg	D	Liu et al. 1999	I-VIII
	RPB2-7R	cccatrgcttgyttrcccat	R	Liu et al. 1999	I-VIII
	RPB2-7F	atgggyaarcaagcyatggg	D	Liu et al. 1999	Pruebas
	RPB2-11R	gcrtggatcttrtcrtcsacc	R	Liu et al. 1999	Pruebas
	CLRPB25F	ctgtttcgaacgctgtttca	D	Yahr et al. 2006	
	CLRPB27R	cgcatccacgtattcaacaa	R	Yahr et al. 2006	
	RPB2dRaq	gctgctaagtctaccat	D	ARTÍCULO III	II-VIII
	RPB2rRaq	atcatgcttggaatctc	R	ARTÍCULO III	I-VIII
rpb1	gRPB1-A	gadrtgtccdggdcattttgg	D	Stiller & Hall 1997	Pruebas
	fRPB1-C	cngcdatntcrttrtccatrta	R	Matheny et al. 2002	Pruebas
mtSSU	mSSU1	agcagtgaggaatattggtc	D	Zoller et al. 1999	Pruebas
	mSSU3R	atgtggcacgtctatagccc	R	Zoller et al. 1999	Pruebas
mtLSU	ML3A	gctggttttctgcgaaacctatata ag	D	Printzen 2002	II
	ML4A	gttagtttgccgagttccttaatg	R	Printzen 2002	II
	ML4	gaggataatttgccgagttcc	D	White et al. 1990	II
cox1	5959F	tcttaacgttgctgtatgctg	D	Printzen & Ekman 2003	I
	6711R	gaaccgaaactagtagaaccata	R	Printzen & Ekman 2003	I
β-tubulina	B42F	cttggtctccatgaaggagg	D	Thon & Royse 1999	Pruebas
	B41R	ctggtactgctggtactcg	R	Thon & Royse 1999	Pruebas
ef1α	CLEF3F	ggcaaaggctccttcaagt	D	Yahr et al. 2006	III-VII
	CLEF3R	gccaataccaccgatcttgt	R	Yahr et al. 2006	III-VII
IGS rDNA	CNL12	gtgaacgcctctaagtcag	D	Arora et al. 1996	Pruebas
	5SAr	cagagtcctatggccgtggat	R	Anderson & Stasovski 1992	Pruebas
	IGSf	tagtggccgwtrgctatcatt	D	Wirtz et al. 2008	V, VII y VIII
	IGSr	tgcatggcttaatctttgag	R	Wirtz et al. 2008	V, VII y VIII
GAPDH	GPD1LM	attggccgcatcgtcttccgcaa	D	Myllys et al. 2003	Pruebas
	GDP2ML	cccactcgttgtcgtacca	R	Myllys et al. 2003	Pruebas

CLONACIÓN

Algunas de las secuencias obtenidas presentaban cromatogramas con picos dobles y fue necesario utilizar la clonación. Este método también se empleó en aquellas muestras cuya amplificación fue difícil y que, una vez purificadas, mostraban una concentración de DNA menor de 20 ng/ml, por lo que no se podían enviar a secuenciar directamente.

Para la clonación se empleó el kit pGEM®-T Easy Vector Systems (Promega, Madison, WI, USA) siguiendo las instrucciones del fabricante. El vector, después de incluir el inserto, se utilizó para transformar las células competentes de *Escherichia coli,* cepa JM109. Las células fueron cultivadas en placas Petri con medio LB (Bacto®-triptona 1%, extracto de levadura 0,5%, NaCl 0,5 % y agar 1,5%) que contenían ampicilina (100 µg/ml), IPTG (0,5 mM) y X-Gal (80 µg/ml) durante 12 a 14 horas a 37 ºC. Se seleccionaron entre 3 y 10 colonias blancas (transformadas) bajo la lupa binocular. A partir de este punto, dependiendo de los medios disponibles y de la rapidez requerida, se utilizó uno de los dos métodos descritos a continuación. En el primer método, las colonias seleccionadas se ponían a crecer en 3 ml de medio LB líquido con ampicilina durante 18 horas. Posteriormente, 2,5 ml se purificaban mediante el Kit QIAprep Spin Miniprep Kit (QIAGEN, Alemania). Para comprobar que las colonias seleccionadas contenían el inserto y no eran un falso positivo, 2 µl del plásmido purificado se digirieron con la enzima Eco RI (cuyas secuencias diana flanquean la zona de inserción en el plásmido). La reacción de digestión contenía 15,4 µl de agua estéril, 0,6 µl de Eco RI (15 u/µl), 2 µl del tampón 10x y 2 µl del plásmido purificado. Esta reacción se incubaba durante 2 h a 37 ºC. Después, 10 l de producto de la digestión se corrían en un gel de agarosa al 2% (7x10 cm; 100 V 30 minutos), lo que permitía visualizar el vector y el inserto.

En el segundo método, mediante un palillo esterilizado se picaban las colonias y se utilizaban como DNA molde para una PCR, en la cual los cebadores utilizados eran los cebadores específicos del plásmido T7-promoter y SP6. Todos los plásmidos amplificados se purificaban con ExoSap; luego se enviaban a secuenciar del mismo modo que las muestras obtenidas por PCR.

ANÁLISIS FILOGENÉTICOS

La edición de las dos cadenas secuenciadas (la directa y la reversa) y la obtención de la secuencia consenso se realizó con el programa SEQUENCHER (Gene Codes Corporation, Inc). A continuación, para confirmar que las secuencias obtenidas pertenecían al género *Cladonia* y no eran contaminaciones, se hizo una búsqueda en el BLAST (Altschul et al. 1997) del National Center for Biotechnology Information (NCBI: http://www.ncbi.nlm.nih.gov/). Cuando se realizó el BLAST se comprobó que varias de ellas pertenecían al alga. Estas secuencias fueron descartadas.

Alineamientos de secuencias

Los alineamientos de las secuencias obtenidas se realizaron de forma manual con los programas SEQAPP (Don Gilbert, Indiana University) o SE-AL v2.0a11 (Rambaut 2002). Además de las secuencias nuevas obtenidas durante el desarrollo de esta tesis, se incluyeron secuencias homólogas procedentes del NIH/NLM/NCBI que se indican en cada uno de los artículos. Se tuvieron en cuenta las transiciones y las transversiones a la hora de hacer los alineamientos. En los alineamientos, los "gap"

se indicaron como "-" y los nucleótidos ambiguos fueron marcados como "N". Sólo en el ARTÍCULO IX los alineamientos se realizaron de forma automática con el programa MAFFT (Katoh et al. 2005) y revisados manualmente.

La elección del grupo externo en cada uno de los casos también se basó en el estudio de Stenroos et al. (2002). Se intentó seleccionar taxones no pertenecientes al grupo de estudio, pero que no fuesen filogenéticamente muy alejados de este.

Métodos de reconstrucción de los árboles filogenéticos

Los alineamientos de los distintos loci se utilizaron para construir los árboles filogenéticos. Se emplearon métodos basados en caracteres: el método de máxima parsimonia, el método de máxima verosimilitud y el método de inferencia bayesiana.

El método de **máxima parsimonia** busca un árbol cuya topología explica los datos con el menor número de cambios (sustituciones). Uno de los problemas más importantes de este método es el conocido como atracción de ramas largas, es decir que taxones divergentes tienden a agruparse juntos.

El método de **máxima verosimilitud** examina diferentes topologías, buscando aquella que maximice la probabilidad de los estados de los caracteres bajo un modelo evolutivo determinado.

Para ambos métodos (máxima parsimonia y máxima verosimilitud) se utilizó como medida de apoyo el método de **bootstrap** (Felsenstein 1985). Este método consiste en hacer réplicas de la matriz de datos original, introducir cambios aleatorios en dichas réplicas y realizar una reconstrucción filogenética de cada una de ellas. Como medida de fiabilidad de un clado se utiliza el porcentaje de veces que aparece en los análisis realizados a partir de las réplicas. Se considera que un clado tiene un alto apoyo cuando el valor de bootstrap es superior al 70% (Hillis & Bull 1993). En los análisis de bootstrap para los análisis de parsimonia se utilizó la opción "fast-step" con 10.000 réplicas (ARTÍCULOS I, II, III, IV), o bien la opción heurística con 1000 réplicas (ARTÍCULOS V, VI, VII, VIII.).

El método de **inferencia bayesiana** emplea el concepto de verosimilitud, pero busca una distribución de probabilidad de árboles. La probabilidad de cada árbol se calcula mediante el teorema de Bayes y se denomina probabilidad posterior. Las probabilidades posteriores son calculadas por exploración del espacio de árboles mediante las cadenas de Markov Monte Carlo (MCMC). Este método comienza con la simulación de un estado de parámetros al azar, proponiéndose después un nuevo estado (conjunto de parámetros). Si los valores del nuevo estado son mejores que los anteriores, este se acepta y se propone uno nuevo. Si el resultado del nuevo estado es peor, se acepta con una probabilidad proporcional al empeoramiento producido. Este procedimiento se repite numerosas veces (generaciones). El número de generaciones empleadas en los análisis realizados en este trabajo fue 2.000.000 (ARTÍCULO I, II, III), 6.000.000 (ARTÍCULO I), 10.000.000 (ARTÍCULO VI), o 20.000.000 (ARTÍCULO IV, V, VII, VIII). El número de cadenas utilizadas fue 12 (ARTÍCULO I, II, III) o 4 (ARTÍCULO IV, V, VI, VII, VIII). Al igual que los análisis de máxima verosimilitud, los análisis bayesianos requieren que se le indiquen el modelo evolutivo.

Los modelos evolutivos para ejecutar los análisis bayesianos fueron seleccionados mediante MrModeltest 2.2 (Nylander 2004) bajo el criterio IAC (Akaike Information Criterion). Este criterio compara simultáneamente todos los modelos. La verosimilitud de cada modelo es penalizada por una función del número de parámetros libres en el modelo. El IAC es un estimador asintóticamente imparcial

de la cantidad de información de Kullback-Leibler, que mide la distancia esperada entre el modelo verdadero y el estimado.

Análisis de redes de haplotipos

Los métodos basados en **redes de haplotipos** se desarrollaron para analizar la variación genética intraspecífica (Posadas & Crandall 2002), pero se ha demostrado que son útiles en la delimitación de especies. Existen diferentes métodos de redes de haplotipos. En el ARTÍCULO VIII se utiliza un método basado en parsimonia estadística (Templeton 1992), el cual primero estima el máximo número de diferencias entre los haplotipos como resultado de sustituciones simples (es decir aquellas que pueden atribuirse a mutaciones únicas en cada posición de la secuencia). A continuación los diferentes haplotipos se van conectando.

Determinación de la congruencia entre loci

El test ILD (incongruence length difference), propuesto por Farris et al. (1994), ha sido empleado por muchos autores para determinar si las matrices de datos eran congruentes y si por tanto se podían combinar. Sin embargo, varios autores han encontrado que este test puede indicar incongruencias incluso cuando no existen (Yoder et al. 2001; Barker & Lutzoni 2002). En esta tesis, por tanto, allí donde este test ha sido utilizado (ARTÍCULO I, II, III), se ha considerado como nivel de significación $P < 0.001$, tal y como recomienda Cunningham (1997). En algunos casos no se ha utilizado como medida de combinabilidad, sino como una medida relativa de la contribución de los diferentes conjuntos de muestras a las incongruencias (ARTÍCULO V).

Durante el desarrollo de este trabajo, el test de combinabilidad más utilizado (ARTÍCULOS IV-VIII) es el propuesto por Lutzoni et al. (2004). Este método considera que existen incongruencias si en el análisis filogenético de un gen aparece un clado con un apoyo del 75% de bootstrap o superior, y las muestras que forman dicho clado en el análisis filogenético de otro gen forman parte de otro clado con apoyo superior al 75% de bootstrap.

Análisis multiloci

Cuando para una determinada muestra no se obtuvo secuencia para todos los marcadores seleccionados, la muestra no fué incluida en los análisis multiloci (ARTÍCULOS I-V, VIII). Sin embargo, en los ARTÍCULOS VI y VII, de los loci que no se obtuvo secuencia, esta se remplazó por N (datos perdidos), debido a que la resolución de los marcadores restantes era muy buena. Para los análisis multiloci se emplearon los mismos métodos de reconstrucción filogenética que para los análisis de un solo locus: máxima parsimonia (ARTÍCULOS I-VIII), máxima verosimilitud (ARTÍCULOS I, IV, V, VI, VII, VIII) e inferencia bayesiana (ARTÍCULOS I-VIII).

Evaluación de la topología de los árboles

Los tests de contraste de hipótesis, o evaluación de la topología de los árboles, se diseñaron para tener una medida de confianza en la topología de los árboles originados por los diferentes análisis filogenéticos, excluyendo así que la topología sea resultado de errores en la estima filogenética. Durante el desarrollo de este

trabajo se emplearon tres diferentes tests: el test de Kishino-Hasegawa (1989) en el ARTÍCULO II, el test de Shimodaira-Hasegawa (SH) (Shimodaira and Hasegawa 1999) en los ARTÍCULOS I, V, VII, VIII, y el test Expected Likelihood Weight (ELW) (Strimmer and Rambaut 2002), en los ARTÍCULOS V, VII, VIII.

DISTANCIAS GENÉTICAS Y ANÁLISIS DE BARCODING

Las distancias genéticas son una medida de la divergencia genética entre los taxones. En general, se estiman en base a un modelo evolutivo. Las distancias genéticas (ARTÍCULO VII) se calcularon con objeto de tener una medida más de apoyo a los resultados filogenéticos y para identificar posibles complejos de especies (Del Prado et al. 2010). También se emplearon para evaluar la utilidad de cada uno de los marcadores para la identificación de especies en *Cladonia* (ARTÍCULO IX).

El **análisis de PCI** (Probability of Correct Identification) es un método que utiliza las distancia genéticas entre cada par de muestras para calcular la probabilidad de monofilia de una especie (Suwannasai et al. 2012). Tal y como se indica en Schoch et al. (2012), dado un alineamiento, primero se calculan las distancias genéticas "p" entre los pares de secuencias. A continuación, para cada especie se obtiene el máximo de distancia entre los especimenes de dicha especie ("sequence diameter" de la especie). Basándose en esa distancia máxima, la correcta identificación de una especie se produce si, para cada una de sus muestras, ningún especimen de otra especie dista de esa muestra menos del "sequence diameter". El valor de PCI representa el porcentaje de especies correctamente identificadas, de acuerdo con el proceso anterior. Este análisis se realizó para evaluar que región génica era la idónea para ser propuesta como un segundo código de barras (barcode) en *Cladonia* (ARTÍCULO IX).

El test de **Wilcoxon** es una prueba no paramétrica que compara si existen diferencias entre dos conjuntos de datos, estando los valores pareados. Se utilizó en el ARTÍCULO IX para comparar las distancias genéticas intraespecífica e interespecífica de los marcadores genéticos estudiados.

ANÁLISIS ESTADÍSTICOS

Los análisis estadísticos empleados en este trabajo están detallados en cada uno de los capítulos. Estos análisis se emplearon para estudiar la variación de los caracteres fenotípicos. Aquí se menciona de forma breve el objetivo de cada uno de los análisis. Los análisis de **tablas de contingencia** (ARTÍCULO III) persiguen analizar las relaciones de dependencia o independencia de dos variables categóricas. Los análisis de **ANOVA** (ARTÍCULO I y III) analizan si existen diferencias entre las medias de las variables relativas a dos o más poblaciones de datos, bajo las condiciones de normalidad de varianza homogénea de las poblaciones. Los **análisis de componentes principales** (ARTÍCULO I, V y VIII) son una clase de análisis multivariante cuyo objeto es reducir el número de variables, originando unas variables nuevas que son combinaciones lineales de las variables originales; el objetivo de este análisis es simplificar los problemas, aunque se pierda una pequeña cantidad de información.

COMPENDIO DE PUBLICACIONES

LISTA DE ARTÍCULOS

Esta memoria doctoral está basada en los siguientes artículos:

I **Pino-Bodas, R.**, Martín, M.P. & Burgaz, A.R. 2010. Insight into the *Cladonia convoluta-C. foliacea* (Cladoniaceae) complex and related species, revealed through morphological, biochemical and phylogenetic analyses. *Systematics and Biodiversity* **8**: 575–586.

II **Pino-Bodas, R.**, Martín, M.P. & Burgaz, A.R. 2012. *Cladonia subturgida* and *C. iberica* (Cladoniaceae) form a single, morphologically and chemically polymorphic species. *Mycological Progress* **11**: 269–278.

III **Pino-Bodas, R.**, Burgaz, A.R. & Martín, M.P. 2010. Elucidating the taxonomic rank of *Cladonia subulata* versus *C. rei* (Cladoniaceae). *Mycotaxon* **113**: 311–326.

IV **Pino-Bodas, R.**, Burgaz, A.R., Martín, M.P. & Lumbsch H.T. 2012. Species delimitations in the *Cladonia cariosa* group (*Cladoniaceae*, Ascomycota). *The Lichenologist* **44**: 121–135.

V **Pino-Bodas, R.**, Burgaz, A.R., Martín, M.P. & Lumbsch H.T. 2011. Phenotypical plasticity and homoplasy complicate species delimitation in the *Cladonia gracilis* group (Cladoniaceae, Ascomycota). *Organisms, Diversity and Evolution* **11**: 343–355.

VI **Pino-Bodas, R.**, Ahti, T., Stenroos, S., Martín, M.P. & Burgaz, A.R. 2012. *Cladonia conista* and *C. humilis* (Cladoniaceae) are different species. *Bibliotheca Lichenologica* **108**: 159–174.

VII **Pino-Bodas, R.**, Ahti, T., Stenroos, S., Martín, M.P. & Burgaz, A.R. 2012. Multilocus approach to species recognition in the *Cladonia humilis* complex (Cladoniaceae, Ascomycota). *American Journal of Botany* (en revisión).

VIII **Pino-Bodas, R.**, Burgaz, A.R., Martín, M.P. & Lumbsch H.T. 2012. Molecular data do not support current circumscription of *Cladonia furcata* and *C. subrangiformis* (Cladoniaceae). Manuscrito inédito.

IX **Pino-Bodas, R.**, Martín, M.P., Burgaz, A.R. & Lumbsch H.T. 2012. Species delimitation in *Cladonia* (Ascomycota): a challenge to the barcoding philosophy. *Molecular Ecology Resource* (en revisión).

I

Insight into the *Cladonia convoluta*-*C. foliacea* (Cladoniaceae) complex and related species, revealed through morphological, biochemical and phylogenetic analyses

ARTÍCULO I

Insight into the *Cladonia convoluta-C. foliacea* (Cladoniaceae) complex and related species, revealed through morphological, biochemical and phylogenetic analyses.

Raquel Pino-Bodas, María P. Martín & Ana Rosa Burgaz

Systematics and Biodiversity (2010) 8: 575-586

Los límites entre las especies del género de líquenes *Cladonia* no son, en general, fáciles de establecer, debido a que cada especie exhibe una gran variedad morfológica. *Cladonia convoluta* y *C. foliacea,* dos taxones comúnmente aceptados muy semejantes entre sí, tienen un talo primario escuamuloso muy desarrollado y en raras ocasiones desarrollan podecios. El carácter que tradicionalmente se ha usado para distinguirlos es el tamaño de las escuámulas, mayores en *C. convoluta* que en *C. foliacea*. Sin embargo, en la Península Ibérica se han encontrado talos intermedios, resultando difícil decidir de cuál de las dos especies se trata. *Cladonia convoluta,* en general, se asocia a sustratos calcáreos, mientras que *C. foliacea* crece sobre suelos ácidos. En este artículo, para dilucidar el rango taxonómico de estos taxones, se han analizado caracteres moleculares (ITS rDNA, *rpb2* y *cox1*), químicos y morfológicos. Nuestros análisis se han llevado a cabo con material procedente de varios países europeos. También se han estudiado otras especies relacionadas desde el punto de vista morfológico por tener un talo primario muy desarrollado, como C. *firma* (incluida en la antigua subsección *Foliosae* junto con *C. convoluta* y *C. foliacea*), *C. cervicornis* y *C. pulvinata* (taxón que tradicionalmente se había subordinado a *C. cervicornis*). A partir de 71 secuencias de ITS rDNA, 33 de *rpb2* y 39 de *cox1* se realizaron análisis de máxima parsimonia, máxima verosimilitud y bayesianos. Los resultados de los análisis de componentes principales mostraron una variación continua, es decir no se distinguieron diferentes grupos entre los especímenes de *C. convoluta* y *C. foliacea*. Los análisis filogenéticos tampoco separaron los

especímenes de las dos especies en dos clados monofiléticos. Los contrastes de hipótesis realizados también rechazaron la monofilia de estas dos especies. Sin embargo, *C. firma, C. cervicornis* y *C. pulvinata* formaron tres grupos monofiléticos diferentes. En cuanto a las relaciones filogenéticas entre los taxones, se encontró que *C. firma* no está estrechamente relacionada con *C. foliacea* y *C. convoluta*, mientras que *C. pulvinata* y *C. cervicornis* sí que están estrechamente relacionadas.

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Research Article

Insight into the *Cladonia convoluta-C. foliacea* (Cladoniaceae, Ascomycota) complex and related species, revealed through morphological, biochemical and phylogenetic analyses

RAQUEL PINO-BODAS[1,2], MARÍA P. MARTÍN[1] & ANA R. BURGAZ[2]

[1]Departamento de Micología, Real Jardín Botánico CSIC, Plaza de Murillo 2, 28014 Madrid, Spain
[2]Departamento de Biología Vegetal I, Facultad de Ciencias Biológicas, Universidad Complutense de Madrid, 28040 Madrid, Spain

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Species boundaries are often not easy to establish in lichens of the genus *Cladonia*, where each species displays a great morphological variability. *Cladonia convoluta* and *C. foliacea* are two commonly accepted, morphologically similar lichen taxa. Molecular (nuclear ITS rDNA, *rpb2* partial gene, *cox1* partial gene), chemical and morphological characters were used to elucidate the taxonomic rank of these taxa. Our analyses were carried out on material from several European countries. Other related species, viz. *C. firma*, *C. cervicornis* and *C. pulvinata* were also studied. Maximum Parsimony, Maximum Likelihood and Bayesian analyses were based on 71 ITS, 39 *cox1* and 33 *rpb2* sequences. Neither morphological characters nor phylogenetic analyses gave evidence to delimit two taxa in the *C. convoluta*/*C. foliacea* complex; however *C. firma*, *C. cervicornis* and *C. pulvinata* formed three distinct monophyletic groups.

Key words: *Cladonia*, lichen, ITS rDNA, *rpb2*, *cox1*, phylogeny, taxonomy

Introduction

Lichens show a great variability in the characters used for taxonomy. The lichen genus *Cladonia* Hill ex Browne comprises more than 400 species worldwide. They are included in *Cladoniaceae*, a family of lichenized *Ascomycota* (Lumbsch & Huhndorf, 2007). Ahti (2000) noted that the species of *Cladoniaceae* are highly variable in gross morphology and this extreme variability is a major problem in identifying these species.

The *Cladonia* species are characterized by having a composite thallus, formed by a primary squamulose or crustose thallus, and a secondary fruticose thallus comprised of erect stalks called podetia. The photobionts are algae of the genus *Asterochloris* (Tschermak-Woess, 1989). The main taxonomical features of the genus *Cladonia* are the morphology and branching of the podetia, the colour of apothecia, the presence and location of vegetative propagules and the chemical composition (Ahti, 2000). The species of *Cladonia* altogether contain about 60 different secondary compounds whose chemical nature is varied: depsides, depsidones, dibenzofurans, terpenes, quinone pigments and higher aliphatic acids (Ahti, 2000).

To study the genus *Cladonia* the taxonomists have usually divided it into several sections and subsections. Mattick (1940) proposed the subsection *Foliosae* (Vainio) Mattick, within the section *Cladonia*, when trying to set up the phylogeny of the genus and fixing infra-genus divisions. This subsection included species with a predominant primary thallus, such as: *C. convoluta* (Lam.) Anders, *C. firma* (Nyl.) Nyl., *C. foliacea* (Huds.) Willd., *C. prostrata* A. Evans, *C. robbinsii* A. Evans, and *C. strepsilis* (Ach.) Grognot. Nevertheless, Huovinen *et al.* (1989) noted that this subsection was rather heterogeneous from a chemical viewpoint. Ahti (2000) reclassified these species according to the chemical compounds they contain. He proposed a new section, *Strepsiles*, to include those taxa that biosynthesize depsides by the β-orcinol pathway (*C. strepsilis* and *C. robbinsii*). However, Stenroos *et al.* (2002) showed that the section *Strepsiles* is not monophyletic (being based on ITSrDNA, β-tubulin gene, morphological and chemical characters). The remaining taxa in subsection *Foliosae* (*C. convoluta*, *C. foliacea*, *C. firma* and *C. prostrata*) contain depsidones and were moved by Stenroos *et al.* (2002) to section *Cladonia* based on morphological, chemical and molecular data.

Correspondence to: Raquel Pino-Bodas. E-mail: rpino@bio.ucm.es

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The phylogenetic relationships of *C. firma* with *C. convoluta* and *C. foliacea* are currently not known.

While *C. firma* is easily distinguishable from *C. convoluta* and *C. foliacea*, the latter two are very similar, both morphologically and chemically. The primary thallus of *C. convoluta* is squamulose and its squamules are slightly bigger than those of *C. foliacea*. Traditionally the length of the squamules was used to differentiate these taxa (Vainio, 1894; Poelt, 1969; James, 2009). Both species contain usnic and fumarprotocetraric acid; although Burgaz & Ahti (1994) reported a second chemotype of *C. convoluta,* containing psoromic acid. Burgaz *et al.* (1993) had problems with their identification because in the Iberian Peninsula intermediate thalli were found. On the other hand, *C. foliacea* is usually associated with acid soils while *C. convoluta* grows on basic ones (Burgaz & Ahti, 1992; Litterski & Ahti, 2004). *Cladonia convoluta* has its distribution center in the Mediterranean region, covering western Eurasia and North Africa (Litterski & Ahti, 2004) while *C. foliacea* is found throughout the same regions but with a wider distribution.

Thus the purpose of this study is to elucidate the taxonomic rank of *Cladonia foliacea* and *C. convoluta*. For this aim the collections of *C. convoluta*, *C. foliacea*, *C. firma* (included in the old subsection *Foliosae*), *C. cervicornis* (Ach.) Flot. and *C. pulvinata* (Sandst.) Van Herk & Aptroot were studied. There were two reasons to include *C. cervicornis* in this work. Firstly, because according to Stenroos *et al.* (2002), it is phylogenetically near *C. convoluta* and *C. foliacea.* Secondly, when podetia are absent, *C. cervicornis* could be mistaken for *C. firma*. *Cladonia pulvinata*, though not included in Stenroos *et al.* (2002), is considered here because of its traditionally admitted close relationship with *C. cervicornis* (James, 2009).

Materials and methods

Materials

In all, 681 specimens were sampled for this study: 178 of *Cladonia convoluta*, 225 of *C. foliacea* (including an isoneotype), 105 of *C. firma,* 163 of *C. cervicornis*, nine of *C. pulvinata* and one of *C. cariosa* (Ach.) Spreng. *Cladonia cariosa* was used as an outgroup, a basal taxon of the clade which contains *C. convoluta* and *C. foliacea* (Stenroos *et al.*, 2002).

Some of the specimens used for this work were freshly collected, while others were herbarium material (maximum 20 years old). The samples selected for morphological analysis and molecular study were chosen trying to encompass their widest variability with regard to morphology, lichen substances they contain, geographical location and altitude of the sampling site. Most of the collections are located at the herbarium MACB Madrid (Table 1).

Table 1. Samples used in the molecular study and their GenBank accession number. Type of substratum: L = limestone, Q = quartzite, G = granite, ST = sandstone, S = sandy spot.

						GenBank Accession Number		
Species	Collection	Soil	DNA Code	CPA Code	Geographic origin	ITS	*cox1*	*rpb2*
C. convoluta	MACB 90622	L	A	15	Spain, Soria	FM205741	—	—
C. convoluta	MACB 90622	L	B	15	Spain, Soria	FM205889	FM208148	FM207567
C. convoluta	MACB 90622	L	C	15	Spain, Soria	FM205890	—	—
C. convoluta	MACB 90517	L	A	1	Portugal, Estremadura	FM205891	—	—
C. convoluta	MACB 41493	L	A	16	Spain, Teruel	FM205892	—	—
C. convoluta	MACB 91687	ST	A	7	Spain, Guadalajara	FM205893	FM208173	FM207574
C. convoluta	MACB 91687	ST	B	7	Spain, Guadalajara	FM211899	—	—
C. convoluta	MACB 90421	Q	A	8	Spain, León	FM205918	FM208174	FM207575
C. convoluta	MACB 90613	ST	A	9	Spain, Lérida	FM205902	FM208152	—
C. convoluta	MACB 90613	ST	B	9	Spain, Lérida	FM205748	—	—
C. convoluta	MACB 92726	L	A	11	Spain, Mallorca	FM205903	FM208166	FM207585
C. convoluta	MACB 92726	L	B	11	Spain, Mallorca	FM205744	—	—
C. convoluta	MACB 92725	L	A	10	Spain, Mallorca	FM205924	FM208176	—
C. convoluta	MACB 92725	L	B	10	Spain, Mallorca	FM205925	FM208177	—
C. convoluta	MACB 90388	ST	A	4	Spain, Cádiz	FM205922	FM208163	—
C. convoluta	MACB 90388	ST	B	4	Spain, Cádiz	FM205746	—	—
C. convoluta	MACB 90499	L	A	46	Spain, Navarra	FM205900	FM208150	—
C. convoluta	MACB 90440	L	A	40	Spain, Barcelona	FM205901	FM208151	FM207584
C. convoluta	MACB 90440	L	B	40	Spain, Barcelona	FM205743	—	—
C. convoluta	MACB 90565	L	A	43	Spain, Granada	FM205886	FM208146	FM207588
C. convoluta	MACB 90565	L	B	43	Spain, Granada	FM205749	—	—

(Continued on next page.)

Table 1. Samples used in the molecular study and their GenBank accession number. Type of substratum: L = limestone, Q = quartzite, G = granite, ST = sandstone, S = sandy spot. *(Continued)*

Species	Collection	Soil	DNA Code	CPA Code	Geographic origin	GenBank Accession Number		
						ITS	*cox1*	*rpb2*
C. convoluta	MACB 90442	ST	A	41	Spain, Cádiz	FM205887	FM208147	FM207586
C. convoluta	MACB 90442	ST	B	41	Spain, Cádiz	FM205888	FM208165	FM207587
C. convoluta	H	L	—	52	Sweden, Öland	FR695859	—	HQ340064
C. convoluta	H	L	—	53	France, Languedoc-Roussillon	FR695861	HQ340072	HQ340065
C. convoluta	H	S	—	54	Italy, Sardinia	FR695860	—	HQ340066
C. convoluta	S L65606	L	—	55	Greece, Crete	FR695862	HQ340073	HQ340067
C. foliacea	MACB 90506	G	A	19	Portugal, Estremadura	FM205894	FM208162	FM207569
C. foliacea	MACB 90506	G	B	19	Portugal, Estremadura	AM922513	—	—
C. foliacea	MACB 90574	G	A	35	Spain, Tarragona	FM205895	FM208143	FM207564
C. foliacea	MACB 90574	G	B	35	Spain, Tarragona	FM205740	—	—
C. foliacea	MACB 90533	G	A	31	Spain, Guadalajara	FM205896	FM208158	—
C. foliacea	MACB 90533	G	B	31	Spain, Guadalajara	FM205897	FM208144	FM207565
C. foliacea	MACB 90503	Q	A	18	Portugal, Alto Alentejo	FM205898	FM208145	FM207566
C. foliacea	MACB 95599	G	A	20	Portugal, Trás-os-Montes	FM205914	FM208159	FM207570
C. foliacea	MACB 95602	—	A	21	United Kingdom, Scotland	FM205915	FM208175	FM207571
C. foliacea	MACB 90414	G	A	30	Spain, La Coruña	FM205919	FM208160	FM207572
C. foliacea	MACB 95600	G	A	17	Denmark, Bornholm	FM205920	FM208161	FM207573
C. foliacea	MACB 91639	Q	A	38	Spain, Ávila	FM205899	FM208149	FM207583
C. foliacea	MACB 91639	Q	B	38	Spain, Ávila	FM205742	—	—
C. foliacea	MACB 90527	G	A	42	Spain, Cuenca	FM205921	—	—
C. foliacea	MACB 90527	G	B	42	Spain, Cuenca	FM205745	—	—
C. foliacea	MACB 90571	L	A	47	Spain, Tarragona	FM205923	FM20864	—
C. foliacea	MACB 90571	L	B	47	Spain, Tarragona	FM205747	—	—
C. foliacea	H	L	—	48	Finland, Varsinais-Suomi, Dragsfjärd	FR695855	—	HQ340068
C. foliacea	H	—	—	49	Finland, Varsinais-Suomi, Korpo	FR695856	—	HQ340069
C. foliacea	H	—	—	50	Italy, Biella	FR695857	HQ340074	HQ340070
C. foliacea	H	S	—	51	Italy, Grosseto	FR695858	—	—
C. cervicornis	MACB 91631	ST	A	—	Spain, Cádiz	FM211897	FM208169	—
C. cervicornis	MACB 91631	ST	B	—	Spain, Cádiz	FM205734	—	—
C. cervicornis	MACB 90738	G	A	—	Spain, Cuenca	FM205904	FM208154	FM207578
C. cervicornis	MACB 90738	G	B	—	Spain, Cuenca	FM205735	—	—
C. cervicornis	MACB 90840	G	A	—	Spain, Madrid	FM205905	FM208170	—
C. cervicornis	MACB 90718	G	A	—	Spain, Cuenca	FM205906	FM208171	—
C. cervicornis	MACB 90718	G	A	—	Spain, Cuenca	FM211898	—	—
C. cervicornis	MACB 91610	S	A	—	Spain, Huelva	FM205916	—	—
C. cervicornis	MACB 91610	S	B	—	Spain, Huelva	FM211900	—	—
C. firma	MACB 91619	G	A	—	Spain, Guadalajara	FM205907	FM208153	FM207568
C. firma	MACB 91619	G	B	—	Spain, Guadalajara	FM205736	—	—
C. firma	MACB 90669	G	A	—	Spain, Madrid	FM205908	FM208167	—
C. firma	MACB 90669	G	B	—	Spain, Madrid	FM205739	—	—
C. firma	MACB 90655	Q	A	—	Spain, Toledo	FM205910	HQ340075	FM207576
C. firma	MACB 90655	Q	B	—	Spain, Toledo	FM205737	—	—
C. firma	MACB 91615	Q	A	—	Spain, Burgos	FM205909	FM208168	FM207577
C. firma	MACB 91615	Q	B	—	Spain, Burgos	FM205738	—	—
C. pulvinata	MACB 91646	G	A	—	Spain, Orense	FM205911	FM208172	FM207579
C. pulvinata	MACB 95597	G	A	—	Portugal, Trás-os-Montes	FM205912	FM208155	FM207580
C. pulvinata	MACB 94339	G	A	—	Portugal, Trás-os-Montes	FM205917	FM208156	FM207581
C. pulvinata	MACB 95598	S	A	—	Spain, Segovia	FM205913	FM208157	FM207582
C. pulvinata	MACB 95598	S	B	—	Spain, Segovia	FM205926	—	—
C. cariosa	MACB 94205	—	—	—	Spain, Gerona	FR695863	HQ340075	HQ340071

Morphological study

Cladonia convoluta and *C. foliacea* were identified using morphological and ecological characters according to Burgaz *et al*. (1993). The samples of *C. firma*, *C. cervicornis* and *C. pulvinata* were identified by morphological and chemical characters according to Van Herk & Aproot (2003) and James (2009).

For each studied specimen (28 samples of *C. convoluta*, 27 of *C. foliacea*, 14 of *C. firma*, 17 of *C. cervicornis* and nine of *C. pulvinata*), the length, width and thickness of three squamules were measured. Hand-cut sections of the squamules were made at 3 mm from the apex, and squash preparations were examined in distilled water and lactophenol cotton blue to measure conidia and ascospores. At least 10 conidia and 10 ascospores were measured when possible. Hand-cut sections of the squamules and ascospores surfaces were examined by light microscopy and scanning electron microscopy. Morphological and anatomical data of *C. convoluta* and *C. foliacea* were analysed by principal component analysis (PCA) by means of the statistical program Statgraphics 5.1 (Statistical Graphics Corp.). The variables included in the analysis were: squamules length, width, thickness and width/length (Vainio, 1894).

Chemical analysis

The lichen substances were extracted by means of acetone (for 10 minutes/10 mg of thallus). The chemical composition of the selected fragments was monitored by thin layer chromatography (TLC) according to standardized procedures (White & James, 1985).

DNA extraction

From each collection two samples were taken (Table 1 example: 'MACB 91687, A' and 'MACB 91687, B'). From every selected sample a small amount (about 10 mg) was taken. Prior to DNA isolation, the lichen chemical substances were extracted by means of acetone (500 μl) for 2 hours, to avoid interference with the extraction and purification of DNA. E.Z.N.A. Fungi DNA Miniprep Kit (Omega Biotech) and DNeasy Plant Mini Kit (Quiagen) kit were used to extract DNA, according to the manufacturer's instructions with slight modifications (Martín *et al.*, 2000). The DNA was dissolved in 200 μl of buffer included in the kit. The concentration of the extracted DNA was quantified using the spectrophotometer RNA/DNA calculator GeneQuant II (Pharmacia Biotech).

DNA amplification

The primers used to amplify the nuclear ITS rDNA from the mycobiont were ITS1F (Gardes & Bruns, 1993) and ITS4 (White *et al.*, 1990) or the newly designed ITSCld (5′ GTAGGCTATACGGCTCATGC 3′)/ITSClr (5′ CTTTCCAACGCGGGATAATA 3′). The amplification cycles were: initial denaturation at 94°C for 5 min; 5 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 1 min; and 33 cycles of 94°C for 30 s, 48°C for 30 s and 72°C for 1 min; with a final extension at 72°C for 10 min (Martín & Winka, 2000). The primer pair RPB2–5F/ RPB2–7cR (Liu *et al.*, 1999) was used for the amplification of *rpb2* partial gene. The amplification cycles were: initial denaturation at 94°C for 5 min; 35 cycles of 95°C for 1 min, 55°C for 2 min and 72°C for 2 min; with a final extension at 72°C for 10 min. The primer pair 5959F-5′ and 6711R-3′ (Printzen & Ekman, 2003) was used for the amplification of *cox1* partial gene. The amplification cycles were: initial denaturation at 94°C for 5 min; six cycles of 95°C for 45 s, 52°C for 45 s and 72°C for 1 min and 45 s; 34 cycles of 95°C for 30 s, 46°C for 30 s and 72°C for 1 min and 45 seconds; with a final extension at 72°C for 10 min.

PCRs were carried out with Ready-to-Go-PCR Beads (GE Healthcare Life Sciences, UK). The volume of reaction was 25 μl for each tube, with 0.4 mM final concentration of primers. The volume of extracted DNA used for the PCR was 1 μl (for initial concentration of extraction >8 μM) or 2 μl (for initial concentration <8 μM). The amplifications were performed in a MJ Research-PTC-200 thermocycler (Bio-Rad). The PCR products were tested by electrophoresis of 5 μl aliquots in a 2% agarose gel (Agarosa D-1 low EEO, laboratorios Pronadisa).

DNA purification and sequencing

Amplification products were purified using the Kit QIAquick Gel Extraction (QIAGEN, Valencia, California, USA). The purified DNA was dissolved in 40 μl of buffer included in the kit. Aliquots of (5 μl) purified DNA mixed with each primer (0.65 μl, 5 μM) used in the amplification were sent to Secugen S. L. (CIB, Madrid, Spain) to get sequences of both chains from each amplimer.

Sequencher^{TM} program (Gene Codes Corporation, Inc, Ann Arbor, Michigan, USA) was used to identify the consensus sequence from the two strands of each isolate. The sequences were input to BLAST (Altschul *et al.*, 1997) of National Center for Biotechnology Information (NCBI: http://www.ncbi.nlm.nih.gov/) in order to make sure that the obtained sequences were not contaminants.

Phylogenetic analyses

The alignments of the obtained sequences and those downloaded from GenBank (EU266114, AF455168, AF455169, AF455170, AF455178, AF455187, AF455227, AF455228, AF4553845, DQ530193, DQ5302206, DQ530207) were made manually using the SeqApp program (Don Gilbert, Indiana University). Alignment gaps were indicated as '-' and ambiguous nucleotides were marked as 'N'. Ambiguous sites were removed from the alignments. The combined

Table 2. Information on Maximum Parsimony analysis, evolutionary model chosen by MrModeltest for each locus and Ln values of ML analyses.

Locus	Total characters	Excluded characters	Variable characters	Informative characters	Tree length	CI	RC	Model	Ln
ITS	580	308	136	84	200	0.7650	0.7108	GTR+I+G	−2127.117
rpb2	915	0	139	104	167	0.8683	0.8334	SYM+G	−2414.878
cox1	776	165	66	51	71	0.9718	0.9651	HKY	−1715.772
combined	2365	0	93	206	403	0.7940	0.7142	GTR+I+G	−5565.905

matrix included only the samples for which the sequences of the three genic regions were available. When the sequences obtained from the two fragments of the thallus were identical, only one of them was included in the phylogenetic analyses. For *rpb2* and *cox1* regions only one of the samples from the same thallus was amplified in the case of both ITS sequences being identical.

The phylogenetic analyses were carried out with PAUP program, version 4.0.b.10 for Macintosh (Swofford, 2003) and MrBAYES 3.1 (Huelsenbeck & Ronquist, 2001). First, Maximum Parsimony (MP) analyses for single genes and for the combined dataset were made, using a heuristic search. Gaps were treated as missing data. For the confidence analysis, bootstrap sampling was applied, with 10 000 replicates, using the fast-step option. The best-fitting evolutionary models were estimated for each region (Table 2) with MrModeltest v2.2 (Nylander, 2004). In Bayesian analyses the posterior probabilities were approximated by sampling trees using Markov chain Monte Carlo (MCMC). Posterior probabilities of each branch were calculated by counting the frequency of trees that were visited during the course of the MCMC analysis. A 2 000 000 generations run for ITS rDNA and *cox1* regions and a 6 000 000 generations run for *rpb2* region and combined dataset (2 millon generations, were not enough for the convergence of the chains), starting at a random tree and employing two runs of Markov chain Monte Carlo with 12 simultaneous chains, were executed. Every 100th tree was saved into a file. Then, the Bayesian analyses were repeated (for all loci and combined dataset), four parallel runs of Markov chain Monte Carlo were performed, each with four chains and 2 000 000 generations for ITS rDNA and *cox1* regions, and 6 000 000 generations for *rpb2* region and combined dataset. The combined dataset was partitioned into three parts, ITS rDNA, *cox1* and *rpb2* for Bayesian analyses. We plotted the log-likelihood scores of sample points against generation time using TRACER 1.0 (http://evolve.zoo.ox.ac.uk/software.html?i=tracer). Ten per cent of initial trees were discarded as 'burn-in' before stationarity was reached. Then, the convergence of the MCMC was evaluated by performing cumulative and sliding window analyses of posterior probability and among-run variability of cumulate and split frequencies using the online application AWTY (Nylander *et al*., 2008). Using the 'sumt' command of MrBAYES, the consensus tree was calculated (for each run), sampled after reaching likelihood convergence to calculate the posterior probabilities of the tree nodes. Phylogenetic trees were drawn using TREEVIEW (Page, 1996) or PAUP. Maximum Likelihood (ML) analyses were performed using PAUP. The models of nucleotide substitution used in ML analyses were the same used in Bayesian analyses. To estimate branch support, 100 bootstrap pseudo-replicates were used.

The statistical congruence among the different regions was tested using ILD test (Farris *et al.*, 1994; Huelsenbeck

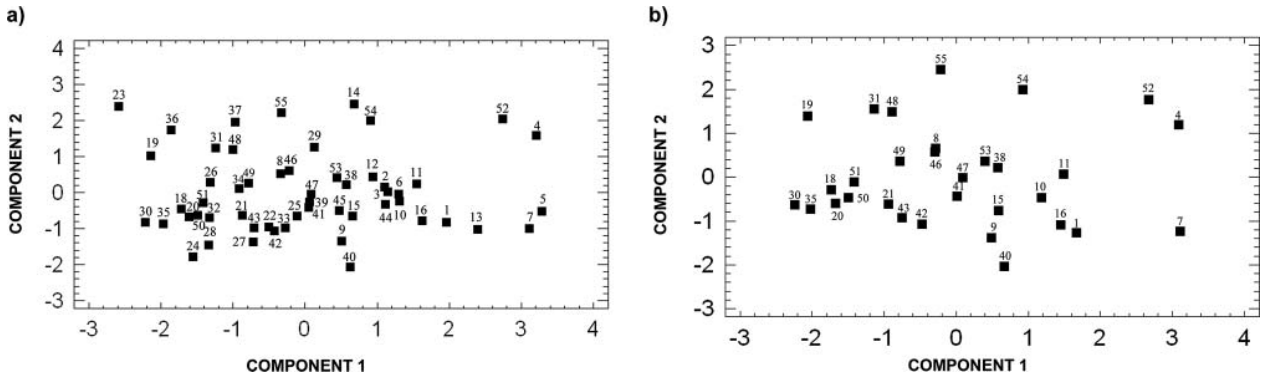

Fig. 1. Results from principal component analyses using morphological and anatomical characters in *C. foliacea* and *C. convoluta*. (a) Analysis with all the specimens. (b) Analysis with the specimens used in molecular analyses.

Table 3. Variation ranges of each studied character in *C. cervicornis*, *C. firma* and *C. pulvinata*.

	Squamules length (mm)	Squamules width (mm)	Squamules thickness (μm)	Conidia length (μm)	Ascospores (μm)
C. cervicornis	(4) 5–12 (24) n = 49	1–2 (4) n = 49	(210) 250–440 (470) n = 49	(5) 6–8 n = 118	(6) 7–13 (15) n = 119
C. firma	(3) 5–17 (20) n = 41	(1) 2–3 (4) n = 41	(213) 300–470 (540) n = 41	(6) 7–9 (10) n = 124	(6) 8–13 (15) n = 56
C. pulvinata	4–9 (14) n = 25	(0.5) 1–2.5 (3.5) n = 25	(188) 190–300 (350) n = 25	(5) 6–8 (9) n = 58	(7) 8–11 (13) n = 18

et al., 1996) carried out with PAUP. The congruence analysis was carried out on only the 29 samples for which the three gene regions were sequenced. The hypothesis that *C. foliacea* and *C. convoluta* are two monophyletic groups was checked by Kishino–Hasegawa (Kishino & Hasegawa, 1989) and Shimodaira–Hasegawa tests (Shimodaira & Hasegawa, 1999) under GTR model. These tests were implemented in PAUP using the combined data set.

Results

Morphology

The results of principal component analysis of morphological variations are shown in Fig. 1. PCA summarizes 84% of the variance of all studied specimens (1A), and 85% of the variance of samples used in molecular analyses (1B). The principal component analyses of morphological characters did not support any group within *Cladonia convoluta* and *C. foliacea*. The analysis shows a continuous morphological variation. No differences were found on squamule sections or on ascospore surface among all studied specimens. On the other hand *C. convoluta* is more commonly associated with limestone soils than *C. foliacea* (Table 1).

The results of morphological and anatomical studies in *C. firma*, *C. cervicornis* and *C. pulvinata* are summarized in Table 3. The squamules length and width of *Cladonia cervicornis* and *C. firma* are similar and higher than in *C. pulvinata.* The conidia of *Cladonia firma* are longer than those of *C. cervicornis* and these ones are similar to those of *C. pulvinata*. The ascospores length and their morphology are similar in the three taxa.

Chemical analyses

Chemical analyses were carried out on 384 specimens. The results are summarized in Table 4. Most of the samples of *C. convoluta* and *C. foliacea* contain fumarprotocetraric and usnic acids, some of them also contain zeorin. Only two specimens with psoromic acid were found (chemotype II of *C. convoluta*). All the screened specimens of *C. cervicornis* contain fumarprotocetraric acid; atranorin, confumarprotocetraric acid; and traces of zeorin appear in some samples. All the screened specimens of *C. firma* contain fumarprotocetraric acid and atranorin, while in some samples traces of zeorin are present. *Cladonia pulvinata* is chemically uniform and all the samples contain psoromic acid; in addition traces of zeorin and more rarely of fumarprotocetraric acid can be found.

Sequence analyses

The DNA was extracted at concentrations that varied between 2 and 25.6 μM. One of the samples of the amplified ITS rDNA region generated two bands of different sizes and both amplimers were cut and processed separately (MACB 90622, B and MACB 90622, C). Seventy-one amplimers of the ITS rDNA region, 41 of *cox1* partial gene and 33 of *rpb2* partial gene were DNA sequenced on both strands. The amplification of one neoisotype of *Cladonia foliacea* was attemped, but it was not achieved for any of the studied markers. The sequences have been deposited in the GenBank (Table 1).

The sizes of the nuclear ITS rDNA sequences are 364–900 bp, those of *cox1* partial gene are 751–886 bp and those of *rpb2* partial gene are 809–1099 bp. The main

Table 4. The secondary substances in the studied taxa. ATR = atranorin, CPH2 = confumarprotocetraric acid, FUM = fumarprotocetraric acid, PSO = psoromic acid, USN = usnic acid, ZEO = zeorin.

	FUM	USN	ATR	PSO	ZEO	CPH2
C. foliacea	109	109	—	—	24	—
C. convoluta	106	106	—	2	14	—
C. firma	69	—	69	—	6	—
C. cervicornis	91	—	4	—	20	16
C. pulvinata	1	—	—	9	1	—

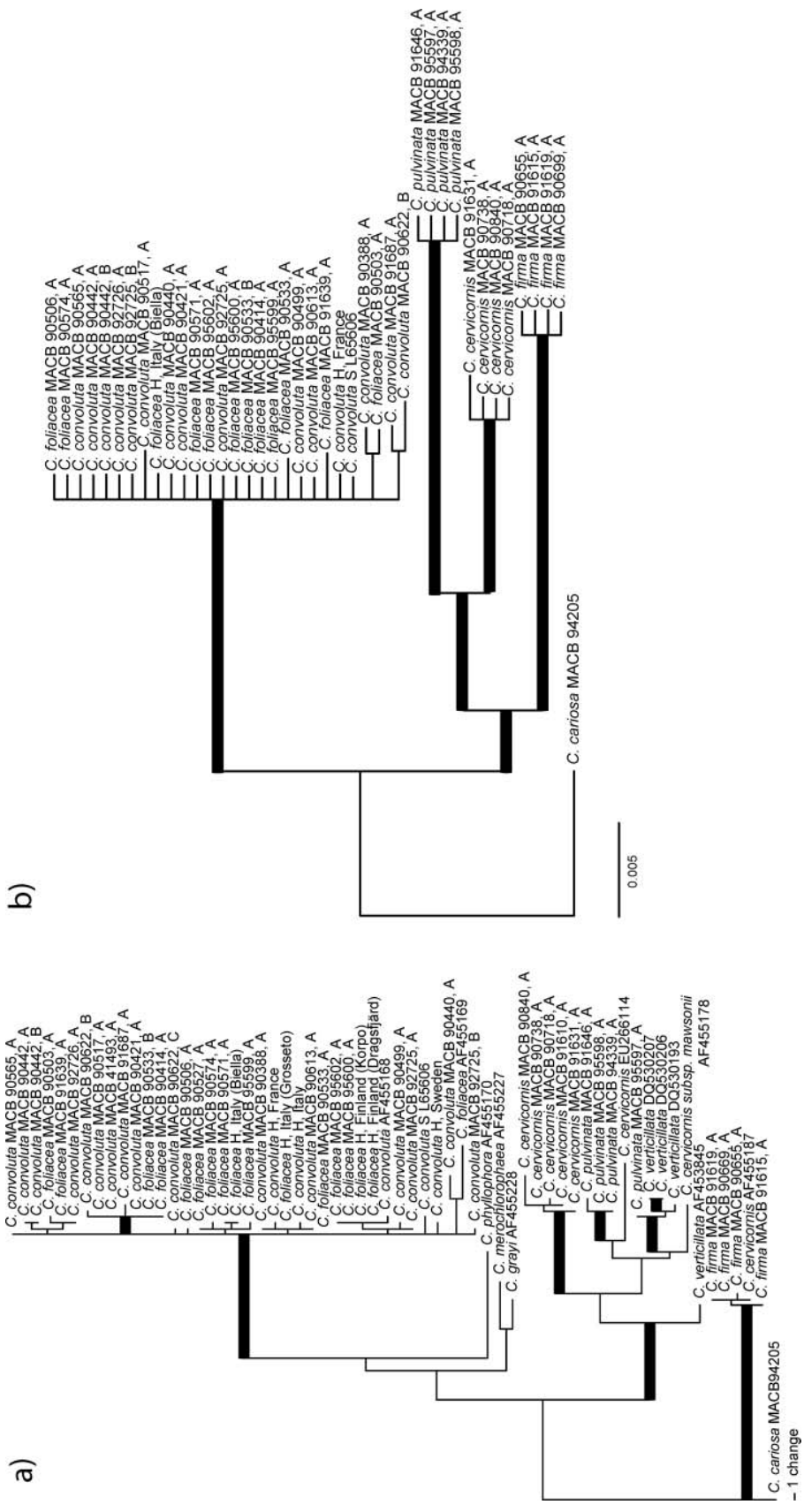

Fig. 2. 50% majority-rule trees of Bayesian analysis of (a) ITS rDNA and (b) *cox1* region. Bold branches indicate bootstrap support value ≥70% for MP and ML and posterior probability ≥0.95 for Bayesian analyses.

difference in size between the ITS rDNA sequences is due to the presence of a group I intron at the 3′ end of the small subunit ribosomal DNA (SSU rDNA) gene inserted after position 1516 (relative to the *Escherichia coli* SSU rRNA gene numbering, hereby named 'S1516 introns' according to Johansen & Haugen, 2001). The intron was found in all the samples of *C. firma* and *C. pulvinata*, 42.8% of *C. foliacea* and 59.2% of *C. convoluta* samples. No sample of *C. cervicornis* has this intron. This intron was not included in the phylogenetic analysis due to its optional occurrence. A 9-nucleotide tandem repeat motif (CAGCTTGCG) was detected in *cox1* partial gene. The repeat number is variable in all species except in *C. pulvinata*, which contains only a single copy. The repeat numbers in *C. convoluta* and *C. foliacea* vary from 1 to 14, in *C. cervicornis* from 10 to 12, and in *C. firma* from 4 to 10. *Cladonia cariosa* (outgroup) does not contain any copy of this motif. A BLAST search of the sequence motif did not detect any homologous counterpart in the databases. This motif was not included in the phylogenetic analysis.

Phylogenetic analyses

Evolutionary models selected by MrModeltest for different regions together with statistical value of parsimony analysis and Ln values of ML analyses are summarized in Table 2. The likelihood parameters of the Bayesian are shown in Appendix 1 (see supplementary material which is available on the Supplementary tab of the article's Informaworld page at http://dx.doi.org/10.1080/14772000.2010.532834). The Tracer and AWTY analyses indicated that the number of generations used in burn-in was sufficient for the chains reached the stationary state and they converged. The phylogenetic trees are shown in Figs 2 and 3. The topologies of MP, ML and Bayesian trees for the ITS rDNA and cox1 regions were similar. According to all the analyses, *C. convoluta* and *C. foliacea* form a monophyletic group with high support. ITS rDNA analyses and *cox1* analyses (Fig. 2) show no subclade inside the *C. convoluta* and *C. foliacea* clade. The *rpb2* analyses give rise to two different topologies (Fig. 3A and B). The first topology (resulting from MP and ML analyses) showed that the samples of *C. foliacea* and *C. convoluta* split into two strongly supported clades, both of them containing samples of both species (Fig. 3A). The tree resulting from the *rpb2* Bayesian analyses groups together all the samples of *C. convoluta* and *C. foliacea* in one clade (Fig. 3B). Within this clade, several strongly supported subclades can be distinguished (Fig. 3B). *Cladonia firma*, *C. cervicornis* and *C. pulvinata* form three monophyletic groups in all analyses (Figs 2 and 3). However, one sample of *C. pulvinata* ('MACB 95597, A') in ITS rDNA analysis is separated from the remaining samples of *C. pulvinata*, joining to *C. verticillata* with strong support. Two sequences of ITS rDNA from GenBank corresponding to *C. cervicornis* appear outside the *C. cervicornis* clade. One of them is nested in the *C. firma* clade and the other appears more related to the *C. pulvinata* clade than to the *C. cervicornis* clade, but with low support.

The partition homogeneity test for the three combined datasets revealed conflict ($P < 0.001$). The comparison of dataset pairs revealed conflict between ITS rDNA and *rpb2* matrix ($P < 0.001$). The reason for this conflict is that the samples of *C. convoluta* and *C. foliacea* which appear as joined in ITS rDNA analysis appear as separated in *rpb2* analysis. For example, the 'MACB 91639, A' sample appears in the same clade that 'MACB 92726, A' in ITS rDNA analysis, but they appear in different clades in *rpb2* analysis. Another example is that of 'MACB 90622, B' and 'MACB 90414, A'. The combined analyses yielded results similar to *rpb2* analyses (Fig. 3C and 3D). The KH and SH tests rejected (P value $= 0.00$ in both analyses) the hypothesis that *C. convoluta* and *C. foliacea* are two monophyletic lineages.

Discussion

The PCA analysis based on morphological characters does not show different groups among the specimens of *Cladonia convoluta* and *C. foliacea*. Due to the great morphological similarity of both taxa, some authors had treated them as having a rank inferior to species, such as variety or subspecies (Vainio, 1894; Suza, 1933; Clauzade & Roux, 1985). The results of some of the phylogenetic analyses of ITS rDNA, *cox1*, *rpb2* regions and combined dataset cannot differentiate two monophyletic groups (Figs 2, 3). On the contrary, MP and ML analyses of *rpb2* region do separate the whole of *C. convoluta*/*C. foliacea* samples into two monophyletic strongly supported clades, but these clades do not correspond with the two morphospecies previously described.

The neotype of *C. foliacea* is from the UK (Burgaz & Ahti, 2009). Although we could not amplify its DNA, we examined the MACB 95602 sample from Scotland and we assume it is similar to the *C. foliacea* type. In *rpb2* analyses, this sample appears within the same clade to which the sample of *Cladonia convoluta* coming from France (Languedoc-Roussillon, H) belongs. France is the country where holotype *Cladonia convoluta* was collected (Burgaz & Ahti, 2009). The incongruities between ITS rDNA and *rpb2* datasets detected by ILD test would invalidate a combined analysis for some authors, but others defend the combined data (Myllys *et al*., 1999), basing their argument on the 'total evidence' (Kluge, 1989). The conflict among loci has been found in plenty of works which use the genealogical concordance phylogenetic species recognition (GCPSR) approach for delimitation of species pairs (Kroken & Taylor, 2001; Buschbom & Mueller, 2006; Velmala *et al.*, 2009). Some authors (Kroken & Taylor, 2001) have chosen to

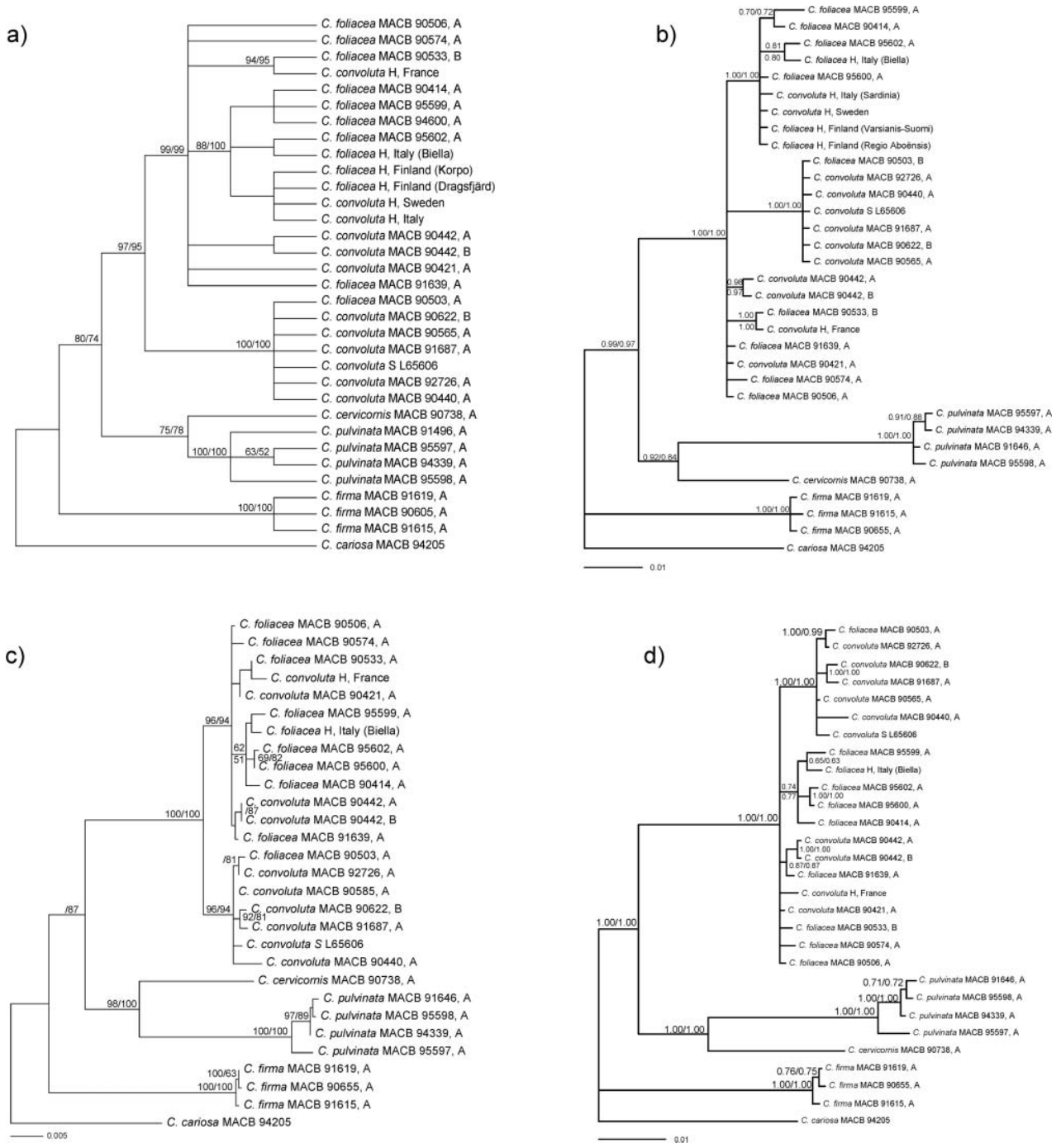

Fig. 3. Phylogeny of the *C. convoluta*/*C. foliacea* complex. (a) Tree for ML analysis of *rpb2* region. Bootstrap values ≥50% for MP/ML at the branches. (b) Tree for the second Bayesian analysis of the *rpb2* region. On the branches, posterior probability values are indicated for both Bayesian analyses. (c) Tree for ML analysis of the combined dataset. Bootstrap values ≥50% for MP/ML at the branches. (d) Tree for the second Bayesian analysis based on the combined dataset. On the branches, posterior probability values for both Bayesian analyses are indicated.

eliminate the taxa which lead to incongruity and combine the loci. The application of this option in the present work would imply the elimination of 13 taxa out of the 20 for which a sequence of the three loci is available.

Van der Niet & Linder (2008) point at several processes, which could lead to some incongruities in gene trees. Some of these are due to biological processes such as hybridization or incomplete lineage sorting. Hybridization has been mentioned as a possible speciation mechanism in some fungi (Staats *et al.*, 2005; Giraud *et al.,* 2008). In the genus *Cladonia* hybridization events have been suggested as an explanation to the polyphyly of *C. pocillum* (Ach.) Grognot and *C. pyxidata* (L.) Hoffm. (Kotelko & Piercey-Normore, 2010).

Incomplete lineage sorting is a common cause of incongruity between closely related species. It is a process by which the tree estimated from a unique locus does not reflect the real phylogenetic history of the taxa, because of the persistence of ancestral polymorphisms, so that individuals of the same species are sorted into different clades. When polyphyly occurs, incomplete lineage sorting could be the cause (Funk & Omland, 2003). The genealogical concordance phylogenetic species recognition (GCPSR) (Avise & Ball, 1990; Taylor *et al.*, 2000) has been the most used method for species delimitation in lichens (Kroken & Taylor, 2001; Ott *et al.*, 2004; Amtoft *et al.*, 2008; Tretiach *et al.*, 2009; Wedin *et al.*, 2009). This method requires the utilization of several gene markers for species delimitation. The species limits lay then on the transition zone between congruences and incongruities. An ever present problem in all the species concepts based exclusively on phylogenetic recognition is their inability to detect incomplete lineage sorting (Templeton, 2001).

Velmala *et al.* (2009) suggested that the presence of incongruities among different loci found in *Bryoria fremontii* Brodo & D. Hawksw. and *B. tortuosa* (G. Merr.) Brodo & D. Hawksw. may be explained by intraspecific recombination. In other cases, it has been suggested that incongruities may be due to incomplete lineage sorting. This explanation is used in *Caloplaca albopruinosa* (Arnold) H. Olivier and *C. variabilis* (Pers.) Müll. Arg. in view of the lack of data to demonstrate the monophyly of the two species (Muggia *et al.*, 2008), and in *Thamnolia* (Nelsen & Gargas, 2009) as a possible cause of the lack of monophyly for the different chemotypes. It is possible that incomplete lineage sorting is responsible for the lack of monophyly in *Cladonia convoluta* and *C. foliacea*. There is also evidence consistent with slow genetic drift in lichens (Printzen *et al.*, 2003), which would contribute to the incomplete lineage sorting. Summing up, the data currently available do not permit the delimitation of two species within the *Cladonia convoluta*/*C. foliacea* complex. In addition, the hypotheses tests rejected the monophyly of each of the two taxa. The morphological differences between the two taxa probably constitute a phenotypical response to different environmental conditions (as substratum type or aridity degree). This fact is supported by the existence of intermediate forms between the two taxa (Burgaz *et al*., 1993; Pino-Bodas, 2006).

As regards *Cladonia pulvinata*, it has for a long time been considered a subspecies of *C. cervicornis* (Ahti, 1980). Regarding the morphology, it is very similar to *C. cervicornis*; but *C. pulvinata* contains psoromic acid and has somewhat narrower podetia (Ahti, 1983). Van Herk & Aptroot (2003) consider *C. pulvinata* to have a species rank. This was based on both morphological characters (squamules smaller than 7 mm, with no deep incisions, rounded lobes, absence of marginal black rhizines, narrow podetia and, at the most, two levels of ramification) and chemical characters (presence of psoromic acid). Our results support this taxonomic change. Samples from the two taxa form independent monophyletic groups strongly supported in the three analyses.

The 'MACB 95597, A' sample groups together with *C. verticillata* in ITS rDNA analysis (Fig. 2), even though this sample contains psoromic acid, never quoted for *C. verticillata*. This sample is morphologically similar to the other *C. pulvinata* samples, though podetia are longer than in the remaining samples (28–32 mm long). It is possible that this sample corresponds to another taxon related to the *C. cervicornis* group. *Cladonia cervicornis* group requires further study, with additional material, to characterize it morphologically and genetically. *Cladonia firma* forms a monophyletic group. However, it neither seems to be closely related to *C. convoluta* and *C. foliacea* nor to the *C. cervicornis* group. On the Iberian Peninsula the ranges of *C. firma* and *C. cervicornis* overlap (see maps in Burgaz & Ahti, 2009), and they have similar ecological demands (Pino-Bodas, 2006). They can be distinguished by chemical substances, thallus size and length of conidia. Possibly *C. firma* is closer to some taxa of the *Helopodium* section (Stenroos, 1988) than to the taxa considered in this paper. Further extensive analyses including a large dataset of Supergroup II taxa (Stenroos *et al.*, 2002) should be performed in order to know which species are most closely related to *C. firma.*

Whereas the *cox1* gene is commonly used as a marker in animals for studying taxonomic relatedness at the species level, it has so far seen little use in fungal analysis (Seifert *et al.*, 2007). In lichens, it has been used to study the intraspecific genetic variability in *Cladonia subcervicornis* (Vain.) Kernst. (Printzen & Ekman, 2003). Unfortunately, our work on the *cox1* gene has provided only a low resolution. The other gene markers studied have more variability than *cox1* gene. The repeat number of the CAGCTTGCG motif could be used in the future for population studies. However, further studies are necessary to check the utility of this *cox1* motif.

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II

Cladonia subturgida and *C. iberica* (Cladoniaceae) form a single, morphologically and chemically polymorphic species

ARTÍCULO II

Cladonia subturgida and *C. iberica* (Cladoniaceae) form a single, morphologically and chemically polymorphic species

Raquel Pino-Bodas, María P. Martín & Ana Rosa Burgaz

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Cladonia subturgida y *C. iberica* constituyen un complejo de especies mediterráneas problemáticas, ya que muestran un gran polimorfismo morfológico. Ambas tienen un talo primario bien desarrollado y podecios que se ramifican en el ápice. Los caracteres usados para distinguir *C. subturgida* de *C. iberica* son el talo primario más pequeño de esta última y su contenido en ácido protoliquesterínico y atranorina, mientras que *C. subturgida* contiene atranorina y ácido fumarprotocetrárico. Sin embargo, los estudios de las Cladoniaceae en la Península Ibérica indicaron que los límites entre ellas no están claros. Otro taxón relacionado es *C. turgida* var. *corsicana,* que con frecuencia se ha confundido con *C. subturgida*. En este artículo se llevó a cabo un estudio cuyo objetivo era delimitar los taxones dentro de este grupo. La variabilidad del grupo se analizó morfológica, química y filogenéticamente. El estudio filogenético se basó en tres loci (ITS rDNA, *rpb2* y mtLSU), analizados mediante máxima parsimonia e inferencia bayesiana. Se analizaron mediante TLC 164 especímenes entre los que se identificaron seis quimiótipos, siendo el más común el que contiene atranorina y ácido protoliquesterínico. Los resultados filogenéticos mostraron un clado monofilético bien apoyado que contenía todos los especímenes de *C. iberica* y *C. subturgida,* quedando demostrado que *C. subturgida* y *C. iberica* constituyen en conjunto una única especie, morfológica y químicamente polimórfica. Los análisis de las secuencias de ITS rDNA mostraron que *C. turgida* var. *corsicana* difiere de *C. turgida* s.s. y no está estrechamente relacionada con ella. Como resultado, se propone una nueva combinación, *C. corsicana*. A pesar de la semejanza morfológica de *C. corsicana* con *C. iberica* y *C. subturgida,* estos taxones no están estrechamente relacionados.

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ORIGINAL ARTICLE

Cladonia subturgida and *C. iberica* (Cladoniaceae) form a single, morphologically and chemically polymorphic species

Raquel Pino-Bodas · María P. Martín · Ana R. Burgaz

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Abstract *Cladonia subturgida* and *C. iberica* constitute the whole of a Mediterranean problematic species, which shows great morphological polymorphism. A study was carried out in order to delimit the extant taxa within this group. The variability of the group was studied morphologically, chemically and phylogenetically. The phylogeny was based on three loci (ITS rDNA, *rpb2* and mtLSU), using Maximum Parsimony and Bayesian analyses. Six chemotypes were identified, the most common one containing atranorin and protolichesterinic acid. Our results prove that *C. subturgida* and *C. iberica* constitute a single, morphologically and chemically polymorphic species. The taxonomic rank of *C. turgida* var. *corsicana* was also studied based on analyses of morphological, chemical and ITS rDNA data. The new nomenclatural combination, *C. corsicana,* is proposed.

Keywords Chemotypes · *Cladonia corsicana* · Phylogeny · Species delimitation

Introduction

The genus *Cladonia* consists of worldwide distributed lichen-forming fungi inhabiting different environments. The species are characterized by a composite thallus, formed by a primary squamulose or crustose thallus, and a secondary fruticose thallus called the podetium. The features of the podetia are very useful in order to distinguish species (Ahti 2000). However, many species of *Cladonia* rarely develop podetia, whereby the identification may be very difficult. One such case in the Mediterranean region is the species pair *Cladonia subturgida* Samp. and *C. iberica* Burgaz and Ahti. The type locality of *Cladonia subturgida* is in Portugal, near the Spanish border, close to the Douro River (Sampaio 1918), while it was later collected in additional areas of the Iberian Peninsula (Burgaz and Ahti 1994; 2009; van den Boom and Giralt 1996; Terrón-Alfonso *et al.* 2000). According to these authors this taxon is characterized by a well developed and elongated primary thallus with a secondary thallus branching at the apices, showing open axils. *Cladonia iberica* was described from the central Iberian Peninsula and currently its distribution includes the western Iberian Peninsula as well as the Canary Islands (Burgaz and Ahti, 2009; Etayo and Burgaz, 1997). The smaller primary thallus and the presence of atranorin and protolichesterinic acid in *C. iberica* distinguish it from *C. subturgida*, which contains atranorin and fumarprotocetraric acid. However, recent studies of *Cladoniaceae* in the Iberian Peninsula (Burgaz and Ahti 1994, 1998, 2009) indicate that more work is required in order to establish the morphological and chemical boundaries between *C. subturgida* and *C. iberica*.

After *Cladonia subturgida* was described, Sampaio (1922) considered it to be a form of *C. turgida* Hoffm. Despite the well-developed primary thallus of *C. turgida*, its podetia are different from *C. subturgida* and so Burgaz and Ahti (1994) regarded *C. subturgida* as a valid species. At the same time, *C. turgida* var. *corsicana* Rondon and Vězda, described from Corsica, was synonymized with *C.*

R. Pino-Bodas (✉) · A. R. Burgaz
Departamento de Biología Vegetal I,
Facultad de Ciencias Biológicas,
Universidad Complutense de Madrid,
E-28040 Madrid, Spain
e-mail: rpino@bio.ucm.es

M. P. Martín
Departamento de Micología, Real Jardín Botánico CSIC,
Plaza de Murillo 2,
E-28014 Madrid, Spain

subturgida (Burgaz and Ahti 1994). On the other hand, Litterski and Ahti (2004) suggested that *C. turgida* var. *corsicana* could be an independent taxon.

Given the current knowledge about the variability and phenotypic plasticity of the *Cladonia* species and noting that most of the traditional infrageneric divisions established by morphological characters are polyphyletic (Stenroos et al. 2002), it is hazardous to make statements about the relationship among species without studying molecular characters. Therefore the aims of this work are: (1) to establish the phylogenetic relationship of *C. subturgida*, *C. iberica* and *C. turgida* var. *corsicana* within the supergroup *Cladonia* (Stenroos et al. 2002) using the ITS rDNA region; (2) to determine whether the morphological affinities among these taxa are correlated with phylogenetic relations; and (3) to characterize *C. subturgida* and *C. iberica* morphologically and chemically.

Materials and methods

Lichen material

This study is based on 188 specimens, 27 being *C. subturgida*, 133 *C. iberica*, 10 *C. turgida* var. *corsicana* and 18 *C. turgida* (including the holotypes of *C. subturgida* and *C. iberica*) The specimens are deposited in the H, MACB, MA-Lichen and PO herbaria.

Morphology and chemistry

Microscopic measurements of the squamules and podetia wall thickness were carried out using freezing microtome. Iodine reactions were performed using Lugol's solution after pre-treatment with 10% potassium hydroxide. Ten ascospores and ten conidia were measured from each thallus. The statistical analyses were done by STATGRAPHICS 5.1 computer program. The variables did not fulfill the normality, and homogeneous variance were analyzed by Kruskal–Wallis test. Thin layer chromatography (TLC) was carried out using solvent systems A and B according to standardized procedures (White and James 1985). When there were several duplicate collections, only one collection was studied by TLC.

DNA extraction and amplification

Twenty collections (Table 1) were selected for molecular study, with an attempt to include all the major morpholog-

Table 1 Samples included in molecular study with GenBank accession number

Specimen code[a]	Taxon	Collection	GenBank no.		
			ITS	rpb2	mtLSU
1IBER	*C. subturgida*	Portugal, Trás-os-Montes, MACB 93695	JF288786	JF288826	JF288805
2IBER	*C. subturgida*	Spain, Jaén, MACB 93537	JF288787	JF288827	JF288806
3IBER	*C. subturgida*	Spain, Segovia, MACB 100441	JF288788	JF288828	JF288807
4IBER	*C. subturgida*	Spain, Madrid, MACB 100442	JF288789	JF288829	JF288808
5IBER	*C. subturgida*	Spain, Burgos, MACB 100443	JF288790	JF288830	JF288809
7IBER	*C. subturgida*	Spain, Tenerife, MACB 99466	JF288796	JF288831	JF288810
TYPEIBER	*C. subturgida*	Spain, Ciudad Real, MACB 49936 (holotype)	JF288795	JF288832	-
1SUBT	*C. subturgida*	Spain, Huelva, MACB 49934	JF288791	JF288822	JF288811
2SUBT	*C. subturgida*	Spain, Cordoba, MACB 100445	JF288792	JF288823	JF288812
3SUBT	*C. subturgida*	Spain, Ciudad Real, MACB 99488	JF288793	JF288824	JF288813
4SUBT	*C. subturgida*	Spain, Salamanca, MACB 100447	JF288794	JF288825	JF288814
COR1	*C. corsicana*	Spain, Sevilla, MACB 100763	JF288797	JF288833	JF288815
COR2	*C. corsicana*	Spain, Sevilla, MACB 101074	JF288798	JF288834	JF288816
COR3	*C. corsicana*	Portugal, Algarve, MACB 101073	JF288799	JF288835	JF288817
COR4	*C. corsicana*	Portugal, Alto Alentejo, MACB 100764	-	JF288836	JF288818
COR%	*C. corsicana*	Spain, Cádiz, MACB 100765	JF288800	JF288837	JF288819
1TURG	*C. turgida*	Canada, Newfoundland, H	JF288801	-	-
2TURG	*C. turgida*	Canada, Ontario, H	JF288802	-	-
-	*C. rangiformis*	Spain, Menorca, MACB 96193	JF288803	JF288838	JF288820
-	*Pycnothelia papillaria*	Spain, Lugo MACB 93242	JF288804	JF288839	JF288821

[a] Specimen code related to morphological identification: *C. iberica* (IBER), *C. subturgida* (SUBT), *C. turgida* var. *corsicana* (COR), *C. turgida* (TURG)

ical and chemical variants in the group. In addition, the holotype of *C. iberica* and the sample 4SUBT, collected near the type locality of *C. subturgida,* were included. The type materials of *C. turgida* var. *turgida* and *C. turgida* var. *corsicana* could not be included.

Previous to the DNA isolation, the secondary lichen substances were extracted by means of acetone for 2 h, so avoiding their obstruction of the subsequent extraction and purification of DNA. E.Z.N.A. Fungi DNA Miniprep Kit (Omega Biotech) and DNeasy Plant Mini Kit (Quiagen) were used to extract DNA, according to the manufacturer's instructions. The DNA was dissolved in 200 μl of buffer included in the kit. The three following loci were amplified: nuclear ITS rDNA using primer ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990), *rpb2* partial gene using two pair of primers RPB25F/RPB27R (Liu et al. 1999) and primer pair RPB2dRaq/RPB2rRaq was used in nested PCR (Pino-Bodas et al. 2010) and mtLSU using ML3A/ML4A (Printzen 2002). PCRs were carried out with Ready-to-Go-PCR Beads (GE Healthcare Life Sciences, UK). The volume of reaction was 25 μl for each tube, with 0.4 mM final concentration of primers. The volume of extracted DNA used for the PCR was 1 μl. The amplification programs were: (1) 94°C for 5 min; 5 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 1 min; and 33 cycles of 94°C for 30 s, 48°C for 30 s and 72°C for 1 min (Martín and Winka 2000) for nuclear ITS rDNA and mtLSU regions, and (2) initial denaturation at 94°C for 5 min; 35 cycles of 95°C for 1 min, 52°C for 30 s and 72°C for 2 min; with a final extension at 72°C for 10 min for *rpb2* region. PCR products were purified using the Kit QIAquick gel extraction (QIAGEN, Valencia, CA, USA). The purified DNA was dissolved in 40 μl of buffer included in the kit. The sequencing reactions were done at he Secugen S.L. (CIB, Madrid, Spain), with the same primers used for the PCR.

Sequence alignments and data analysis

The sequences were edited using Sequencher™ program (Gene Codes, Ann Arbor, MI, USA). The alignments were done manually, using SEQAPP (Don Gilgert) and SE-AL v2.0 (Rambaut 2002) programs. To infer phylogenetic relationships of *C. subturgida*, *C. iberica* and *C. turgida* var. *corsicana* within *Cladonia* supergroup, we constructed an alignment with 72 sequences of ITS rDNA, 63 of which were downloaded from GenBank. A second dataset with sequences of ITS rDNA, *rpb2* and mtLSU for 20 specimens was constructed for molecular characterization of *C. subturgida* and *C. iberica*. *Pycnothelia papillaria* was used as an outgroup in the phylogenetic analyses according to Stenroos et al. (2002). The ambiguous sites were removed of the alignments. Maximun Parsimony (MP) analyses were performed with PAUP 4.0b10 (Swofford 2003) and Bayesian analyses with MrBayes 3.1 (Huelsenbeck and Ronquist 2001) for the separate genes and for the concatenated dataset. MP analyses were done using heuristic search with 500 random addition replicates with TBR Branch-swapping option. Gaps were treated as missing data. Branch robustness was estimated by bootstrap (bs) analysis employing 10,000 replicates, using the fast-step option. MrModeltest (Nylander 2004) was used for selecting the best evolution model for every region according to Akaike criterion. For the first dataset the model selected was SYM+I+G. For the second dataset, the model SYM+G was selected for ITS rDNA and *rpb2* regions; the model HKY+G for mtLSU region; and the model GTR+G for the combined analysis. The Bayesian analyses were performed using these models. The posterior probabilities were approximated by sampling trees using Markov Chain Monte Carlo (MCMC). The posterior probabilities (pp) of each branch were calculated by counting the frequency of trees that were visited during the course of the MCMC analysis. The MCMC was run 2,000,000 generations, with 12 chains starting from the random tree. Every 100th tree was saved into a file. The first 2,000 trees were discarded as burn-in. TRACER 1.0 (http://tree.bio.ed.ac.uk/software/tracer/) was used to plot the log-likehood scores of sample points against generation time. The consensus trees were calculated using the "sumt" command. The congruences among the matrices were carried out with partition homogeneity test (Farris et al. 1994) on PAUP.

The following phylogenetic hypotheses were tested: (1) *C. turgida* var. *corsicana* is not an independent group of *C. turgida*; and (2) *Cladonia iberica* and *C. subturgida* are monophyletic independent groups. The Kishino–Hasegawa (KH) (Kishino and Hasegawa 1989) likehood test was conducted using PAUP to compare the best topology obtained with a constrained tree. The constructed tree topology was done by MacClade v.4.0.5 (Maddison and Maddison 2002). The most parsimonious tree consistent with the constrained tree was found using the heuristic search by PAUP.

Results

Morphology and secondary chemistry

The morphological and anatomical studies revealed that *C. subturgida* and *C. iberica* have significant differences in squamule length, width and thickness (Table 2).

The secondary compounds of 164 specimens (included 7 of *C. turgida* var. *corsicana* and 2 of *C. turgida*) were analyzed. The results of *C. subturgida* and *C. iberica* are

Table 2 Results of statistical analyses. The minimum value corresponds to percentile 10 and the maximum to percentile 90. The absolute maximum and minimum values are in brackets

	C. subturgida	*C. iberica*	*n*	*P* value
Squamules length (mm)	6.5–21(25)	(3)5–14(16)	214	0.000
Squamules width (mm)	1.5–4(5)	(1)1.5–4(4.5)	214	0.025
Squamules thickness (μm)	(175)205–327(365)	(150)187.5–287.5(350)	214	0.000
Podetia length (mm)	(2.5)4–14(20)	(4)6–13(16)	74	0.378
Podetia thickeness (μm)	(125)150–250(260)	(125)157.5–275(337.5)	74	0.673
Coninia legth (μm)	(6)7–9(11)	(6)7–10(11)	718	0.899
Ascospores length (μm)	(10)11–16(19)	(9)10–16(19)	210	0.703

summarized in Table 3. Six different chemotypes were identified. The most common is the chemotype II, present in 116 samples, which contains atranorin and protolichesterinic acid, with zeorin as an accessory substance. The second one in frequency is the chemotype V (fumarprotocetraric and protocetraric acids; zeorin accessory) found in 15 samples. There is no relationship between the geographical origin of the samples and the chemotypes, nor between chemotypes and altitude. All specimens of *C. turgida* var. *corsicana* contain fumarprotocetraric and protocetraric acids, with zeorin and confumarprotocetraric as accessory. The samples of *C. turgida var. turgida* contain atranorin, fumarprotocetraric and protocetraric acids.

Phylogenetic analysis

The DNA concentration ranged from 2–29.6 ng/μl. A total of 54 new sequences have been generated in this work (19 of ITS rDNA, 18 of *rpb*2 and 17 of mtLSU). The aligment of ITS rDNA for testing the phylogenetic relationship of *C. subturgida*, *C. iberica* and *C. turgida* var. *corsicana* contained 72 sequences with 573 unambiguous characters, of which 186 were parsimony informative. The MP analysis resulted in 1,000 equally parsimonious trees with 723 steps, CI=0.4661, RI=0.6754. The Bayesian (Fig. 1) and the MP analyses lead to trees with similar topology. The likelihood parameters of Bayesian analysis are the following [mean values (SD)]: LnL = −41617.334 (0.928), r(AC)=0.04885 ($2.38e^{-4}$), r(AG)=0.178 ($9.56e^{-4}$), r(AT)=0.111 ($3.65e^{-4}$), r(CG)=0.02008 ($9.75e^{-5}$), r(CT)=0.609 ($1.39e^{-3}$), r(GT)= 0.03307 ($1.38e^{-4}$), Base frequencies π(A)=0.223 ($3.333e^{-4}$.), π(C)=0.262 ($2.993e^{-4}$), π(G)=0.247 ($2.782e^{-4}$), π(T)=0.269 ($3.554e^{-4}$), α=0.418 ($2.514e^{-3}$), pinvar=0.239 ($1.38e^{-3}$). In both analyses, the specimens of *C. turgida* var. *corsicana* constituted a strongly supported clade independent on *C. turgida* var. *turgida*. *Cladonia turgida* var. *corsicana* appeared related to a clade consisting of taxa of the *C. furcata* group and *C. humilis* (With.) J. R. Laundon, while *C. turgida* s. str. was included in a clade together with *C. caespiticia* (Pers.) Flörke, *C. cervicornis* (Ach.) Flot., *C. cervicornis* ssp. *mawsonii* (C. W. Dodge) S. Stenroos and Ahti, *C. pulvinata* (Sandst.) Aptroot and van Herk, and *C. subulata* (L.) F. H. Wigg. *Cladonia subturgida* and *C. iberica* constituted a distinct, well-supported clade. This clade formed a highly supported sibling group with *C. rangiformis* Hoffm.

The separate analysis of the ITS rDNA, *rpb*2 and mtLSU for the second dataset (not shown) yielded a similar topology. Two strongly supported monophyletic clades appeared. One clade included all specimens of *C. iberica* and *C. subturgida* and the other contained the *C. turgida* var. *corsicana* specimens. The partition homogeneity test indicated no conflict (*P*=0.29) among the three datasets, thus an analysis based on combined datasets was done. The combined alignment contained as many as 2,580 characters (628 of ITS rDNA, 899 of *rpb*2 and 1053 of mtLSU), 205 out of them being informative for parsimony. The MP analysis generated 500 equally parsimonious trees, 500 steps long, CI=0.9720 and RI=0.9793. The Bayesian analysis was run under the GTR+G sequence evolutionary model (selected by MrModeltest). The values of likelihood parameters resulting from Bayesian analysis are the

Table 3 Chemotypes of *Cladonia subturgida,* including both morphoespecies *C. subturgida* (SUBT) and *C. iberica* (IBER)

ATR atranorin, *PLIC* protolichesterinic acid, *FUM* fumarprotocetraric acid, *PRO* protocetraric acid, *ZEO* zeorin, + presence, ± accesory substance

Chemotype	ATR	PLIC	FUM	PRO	ZEO	No. samples	SUBT	IBER
I	+					2	–	2
II	+	+			±	116	1	115
III	+	+	+	+		15	13	2
IV	+		+	+		6	4	2
V			+	+	±	15	14	1
VI		+	+	+		1	–	1

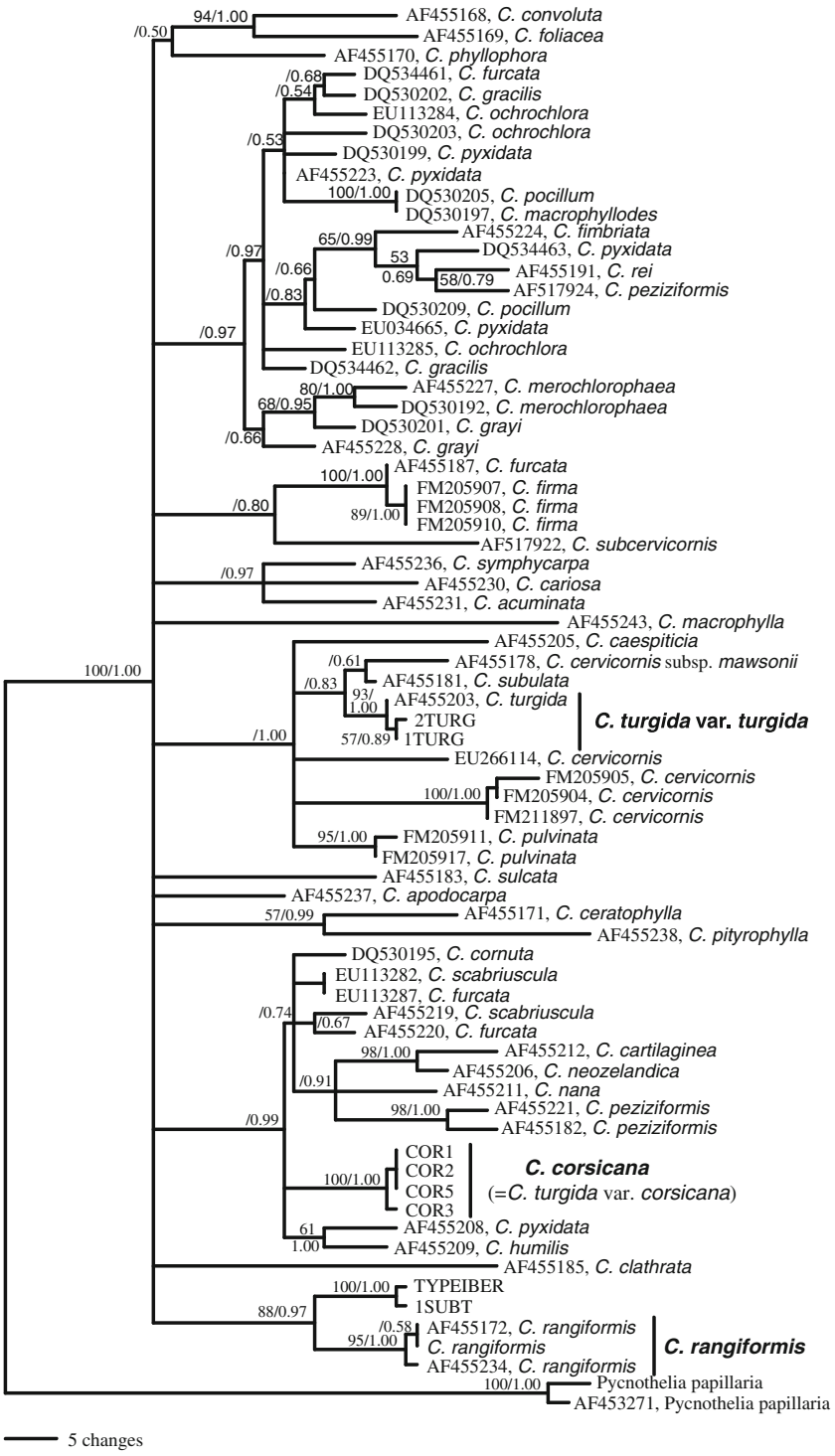

Fig. 1 The 50% majority-rule consensus tree of Bayesian analysis based in ITS rDNA. Bootstrap and posterior probability values indicated at branches

following [mean (SD)]: LnL = −5990.831 ($9.144e^{-2}$), π(A)=0.296 ($1.226e^{-4}$), π(C)=0.199 ($1.047\ e^{-4}$), π(G)=0.221 ($1.055e^{-4}$), π(T)=0.284 ($1.204e^{-4}$), rate matrix r(AC)=0.0621 (0.000182), r(AG)=0.23 (0.000293), r(AT)=0.115 (0.000175), r(CG)=0.0980 (0.0002408), r(CT)=0.397 (0.0003786), r(GT)=0.09052 (0.0001319), gamma shape parameter alpha=0.534 (0.00689). The MP and Bayesian consensus trees did not show conflicts. Figure 2 shows the 50% majority-rule consensus Bayesian tree. Two strongly supported clades appeared: one constituted by all the specimens of *C. iberica* and *C. subturgida* intermixed (bs=99%, pp=1.00), including the *C. iberica* holotype, and another which includes the specimens of *C. turgida* var. *corsicana*.

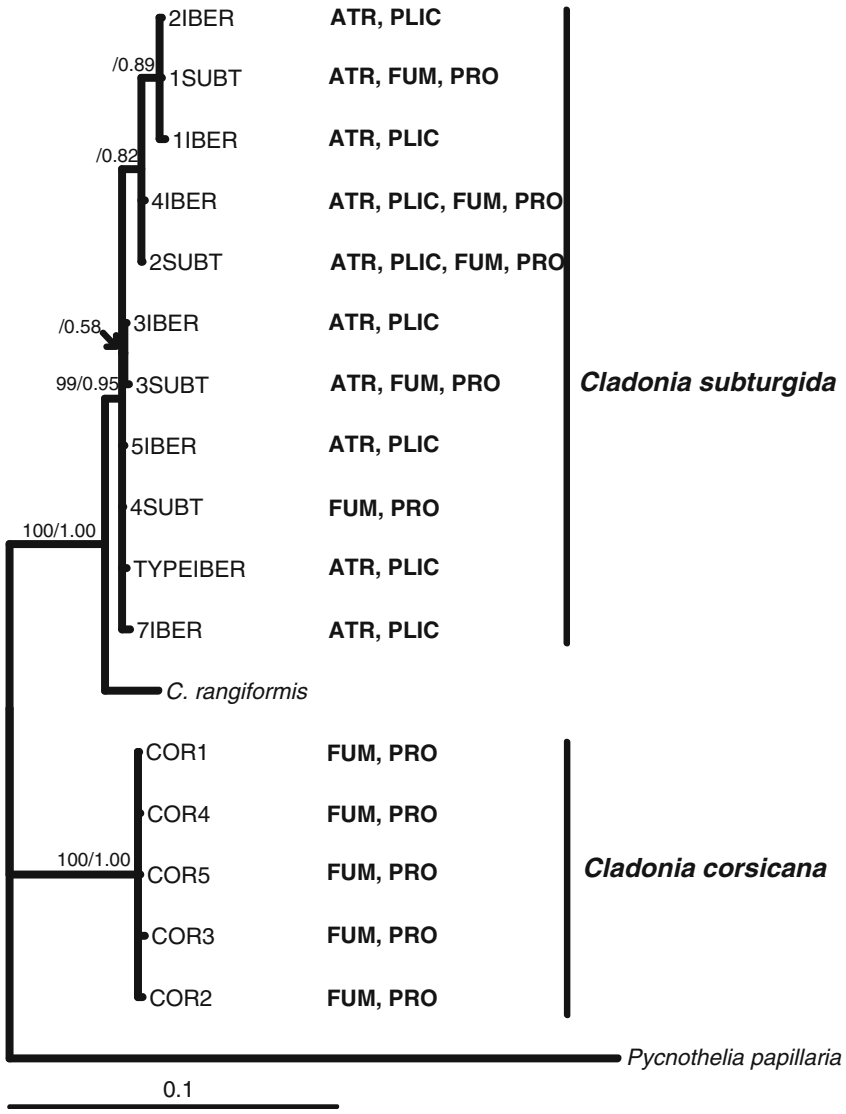

Fig. 2 The 50% majority-rule consensus tree of Bayesian analysis inferred from a three loci. Bootstrap and posterior probabilities values indicated at branches. The chemical substancies detected by TLC is shown on the tree. *ATR* atranorin, *PLIC* protolichesterinic acid, *FUM* fumarprotocetraric acid, *PRO* protocetraric acid, *ZEO* zeorin

The Kishino–Hasegawa test significantly rejected two hypotheses: (1) *C. turgida* var. *corsicana* is not an independent group of *C. turgida* (P=0.00); and (2) the monophyly of *C. subturgida* and the monophyly of *C. iberica* (P=0.00).

Discussion

Molecular data supported the recognition of *C. turgida* var. *corsicana* specimens as a monophyletic entity. This taxon was known from Corsica, Sardinia and the Iberian Peninsula (Poelt and Vězda 1977; Burgaz and Ahti 1992). A revision of the specimens coming from the Iberian Peninsula confirmed that they belong to *C. subturgida;* therefore, *C. turgida* var. *corsicana* was excluded from this area (Burgaz and Ahti 2009). However, the present work again maintains the presence of *C. turgida* var. *corsicana* in the Iberian Peninsula. This conclusion is based on the comparative study of an isotype of this species with newly collected Iberian material. Morphologically, *C. turgida* var. *corsicana* is clearly different from *C. turgida* s. str. Although both taxa have similar primary thallus, their podetia are different. The podetia of *C. turgida* are robust, subcylindrical, with complex patterns of branches and sometimes small cups (Vainio 1887; Thomson 1967), while *C. turgida* var. *corsicana* presents no cups and its podetia are split (Vězda 1970). In addition, *C. turgida* contains atranorin and fumarprotocetraric acid, while *C. turgida* var. *corsicana* produces only fumarprotocetraric acid (Poelt and Vězda 1977). With respect to their distribution, *C. turgida* is a circumboreal species, widespread in Europe, Asia and North America (Litterski and Ahti 2004). Its southern limit in Europe is in northern Italy (Nimis 1993), while *C. turgida* var. *corsicana* is restricted to the Mediterranean area. The phylogenetic study based on ITS rDNA region proves that

the morphological differences between *C. turgida* var. *corsicana* and *C. turgida* are supported by genetic differences. On the other hand, each of these taxa is related to different groups (Fig. 1). *Cladonia turgida* is related to *C. subulata* and the *C. cervicornis* group. In turn, *C. turgida* var. *corsicana* is related to a clade including *C. furcata* and *C. scabriuscula* (Delise) Nyl., among other taxa. These two last taxa were earlier (Ahti 2000) included in the section *Ascyphiferae,* which also included *C. turgida,* but Stenroos et al. (2002) proved that *C. turgida* was not related to the other taxa in this section. In the phylogeny of Stenroos et al. (2002) based on ITS rDNA, β-tubulin gene and morphological and chemical characters, *C. turgida* was related to *C. macrophyllodes* Ahti and Fleig. We consider *C. turgida* var. *corsicana* as not closely related to *C. turgida* s. str.

As noted, the results based on the three studied gene regions (ITS rDNA, *rpb2* y mtLSU) do not support the separation of *C. subturgida* and *C. iberica.* The morphological and chemical differences found are to be interpreted merely as intraspecific variation of a single species. Furthermore, this conclusion is supported by the coincidence of their distribution areas, mainly concentrated in the Iberian Peninsula (although *C. subturgida* has not been collected in Canary Islands), as well as by the fact of having similar ecological requirements. Great intraspecific variation is common in the genus *Cladonia,* which contains several polymorphic species (Ahti 2000). Though the number of chemotypes identified in *Cladonia subturgida* is high, these chemotypes do not form monophyletic groups, and no geographical pattern has been found as for their distribution. Moreover, several chemotypes were found even in the same mat. The holotype of *C. subturgida* itself shows chemical variations. We found that it contains protolichesterinic acid in low concentration and atranorin, while Burgaz and Ahti (1994) found atranorin and traces of fumarprotocetraric acid. Thus, *Cladonia subturgida* is another chemically polymorphic species, one more of the many cases where the chemotypes do not group into monophyletic clades (Buschbom and Mueller 2006; Zhou et al. 2006; Velmala et al. 2009; Ohmura et al. 2008). In the genus *Cladonia*, the presence of several chemotypes within the same species is very common. Some examples are *C. symphycarpa* (Flörke) Fr., *C. subcariosa* Nyl., *C. capitellata* (Hook. f. and Taylor) C. Bab., *C. incrassata* Flörke, etc. (Huovinen et al. 1989, 1990). The chemotype I of *C. subturgida*, only found in two samples, could contain protolichesterinic acid in very low concentration, impossible to detect by TLC. In such a case, chemotype I and chemotype II (the most frequent one) would be the same.

In spite of the fact that *C. subturgida* and *C. rangiformis* are morphologically very different, they appear in our analyses as phylogenetically related, with high support. The primary thallus of *C. subturgida* is persistent, while in *C. rangiformis* the primary thallus disappears as soon as the secondary thallus develops. In addition, the podetia of *C. rangiformis* are more branched than those of *C. subturgida*. Burgaz and Ahti (1994) included *C. subturgida* in sect. *Helopodium*, which included taxa with persistent and big primary thallus, corticate and rarely scyphoid podetia. As for chemical constituents, they contain depsidones of the β-orcinol path (Stenroos 1988; Ahti 2000). However, it has been proved that *Helopodium* is polyphyletic (Stenroos et al. 2002). In this work, the taxa of *Helopodium* section, *C. cariosa* (Ach.) Spreng., *C. symphycarpa* (Flörke) Fr., *C. apodocarpa* Robbins, *C. caespiticia* (Pers.) Flörke, *C. cartilaginea* Müll. Arg., *C. nana* Vain., *C. macrophylla* (Schaer.) Stenh., *C. acuminata* (Ach.) Norrl., *C. ceratophylla* (Sw.) Spreng., *C. pityrophylla* Nyl., *C. sulcata* A. W. Archer and *C. neozelandica* Vain. were included. None of these taxa appears related to *C. subturgida*.

Taxonomy

Cladonia subturgida Samp.

Ann. Acad. Polytechn. Porto 13: 106 (1918); type: Portugal, Beira Alta, Guarda, Barca d'Alva, 13-XII-1916, *G. Sampaio 245* [holotype, PO (!)].

= *Cladonia iberica* Burgaz and Ahti, Nova Hedwigia 59: 430 (1994); type: Spain, Ciudad Real, Puebla de Don Rodrigo, 690 m, on acid soils with *Cistus ladanifer* shrub, 16-VI-1993, *A. R. Burgaz* [holotype, MACB 49936!, isotype, H].

Iconography in Burgaz and Ahti (1994, 2009).

Primary thallus persistent, dominant, squamulose, squamules (3–)6–16(−19) mm long and (1–)1.5–4(−5) mm wide, deeply lobate (up to 70% of their length), upper surface light green to olivaceous, lower surface white, usually pink, or purplish towards tips. Podetia rare, without scyphi, (2.5–)5.5–13(−20) mm tall, (0.7–)1–2.3(−3.5) mm wide, light green, branched at the apices, with open axils. Podetium surface smooth, esorediate, corticated. Apothecia dark brown, common. Ascospores simple, hyaline, (9–)10–16(−19) x (2–)3–4 μm. Pycnidia black, frequent, 310–700 μm long, on upper surface or margins of the squamules, or sometimes on sides of podetia, subglobose, constricted at the base, to cylindrical, sessile or pedunculate. When pedunculate, they usually grow two by two, containg hyaline gelatin. Conidia hyaline, filiform, (6–)7–10 (−11) x 0.5–1 μm.

Anatomy Squamules of primary thallus (125–)187.5–300 (−360) μm thick; cortex (25–)37.5–75(−87.5) μm thick; algal layer (12.5–)25–37.5(−60) μm thick; medulla (75–)112.5–221.5(−270) μm. Podetial wall (125–)150–250 (−337.5) μm; cortex (17.5–)25–37.5(−50) μm; algal layer

(12.5–)20–50(−100) μm; medulla (25–)30–100(−170) μm; stereome (37.5–)50-127(−185) μm.

Chemistry Pd– or Pd+red, K– or K+yellow, C–, UV–. Six known chemotypes: I) atranorin; II) atranorin, protolichesterinic acid and zeorin (inconstant); this is the most frequent chemotype; III) atranorin, protolichesterinic, fumarprotocetraric and protocetraric acids; IV) atranorin, fumarprotocetraric and protocetraric acids; V) fumarprotocetraric and protocetraric acids, with zeorin (inconstant); VI) fumarprotocetraric, protocetraric and protolichesterinic acids.

Habitat and distribution It grows on acid substrates, in pine groves, cork oak (*Quercus suber*) woodlands, holm-oak (*Q. ilex*) woods, *Q. pyrenaica* woodlands, and *Cistus* scrubs, at an altitude of 15 to 1500 m in the western area of the Iberian Peninsula and Canary Islands.

Remarks A highly variable species, both morphologically and chemically. In absence of podetia it can be mistaken to *C. firma* (Nyl.) Nyl. or *C. cervicornis* (Ach.) Flot. (despite these taxa are not phylogenetically related to *C. subturgida,* Fig. 1), which are very common in the Mediterranean region.

Additional specimens examined **PORTUGAL:** *Algarve*: Bensafrim, Sª do Espinhaço de Cao, *A. R. Burgaz* (MACB 99474, 99451); Monchique, Sª de Monchique, Barranco do Banho, *A. R. Burgaz* (MACB 99435); Monchique, Sª de Monchique, subida a Foia, *A. R. Burgaz* (MACB 100464); *Alto Alentejo*: Bencatel, Sª de Ossa, *A. R. Burgaz* (MACB 99465); Evoramonte, *A. R. Burgaz* (MACB 94140, 99448); Pégoes, Monte das Piçarras, *A. R. Burgaz* (MACB 100460, 99443); *Trás-os-Montes*: Izeda, valle del río Sabor, *A. R. Burgaz* (MACB 93871); Lagoa, valle del río Sabor, *A. R. Burgaz* (MACB 93994); Lagoa, valle del río Sabor, *R. Pino-Bodas* (MACB 93695, 93695); Rebordãos, Sª da Nogueira, *A. R. Burgaz* (MACB 93696, 93704, 93872); Rebordãos, Sª da Nogueira, *R. Pino-Bodas* (MACB 99473); *Beira Litoral*: Campieses, Sª de Sicó, *A. R. Burgaz* and *I. Martínez* (MACB 68522). **SPAIN:** *Albacete*: Villapalacios, Sª del Relumbrar, *A. R. Burgaz* (MACB 93536); *Almería*: Rodalquilar, Sª de Cabo de Gata, *A. R. Burgaz* (MACB 99478, 100455); *Ávila*: Navamojada, *A. R. Burgaz* (MACB 100467); Peguerinos, alrededores del campamento "Peñas Blancas", *A. R. Burgaz* (MACB 100461); Piedrahita, *A. R. Burgaz* (MACB 94141); Piedrahita, Pto. de Peñas Negras, *A. R. Burgaz* (MACB 99484); Ramacastañas, *A. R. Burgaz* (MACB 94142); *Badajoz*: Campillo de Llerena, Cortijo "Las Navillas", *E. Fuertes* (MACB 49935); Castuera, "Las Navillas", *E. Fuertes* (MACB 99453); Zahinos, *A. R. Burgaz* (MACB 99441); *Barcelona*: El Brull, P. Nat. del Montseny, coll de Formia, *A. R. Burgaz* (MACB 100449); Montseny, P. Nat. del Montseny, *A. R. Burgaz* (MACB 100448); *Burgos*: Covarrubias, Sª de Covarrubias, *A. R. Burgaz* (MACB 100443, 93613, 99442); *Cáceres*: Casas de Miravete, Pto. de Miravete, *A. R. Burgaz* (MACB 99439); La Calera, Sª de la Palomera, *A. R. Burgaz* (MACB 99421, 99424, 99426); Perales del Puerto, Sª de Gata, *A. R. Burgaz* (MACB 99422, 99429); Robledollano, *A. R. Burgaz* (MACB 99423); Salorino, ribera de los Molinos, *A. R. Burgaz* (MACB 100445, 94143, 99436); Villareal de San Carlos, *A. R. Burgaz* (MACB 99430); Villareal de San Carlos, P. Nat. Sª de Monfragüe, *A. R. Burgaz* (MACB 99428, 99451); *Ciudad Real*: Albaladejo, Sª del Relumbrar, *A. R. Burgaz* (MACB 93540, 93541, 99446); Almodovar del Campo, Sª Umbría de Alcudia, Puerto de San Juan, *A. R. Burgaz et al.* (MACB 99420, 99427, 100465); Piedrabuena, Cerro Navalagrulla, *A. R. Burgaz* (MACB 99432); Puebla de Don Rodrigo, *A. R. Burgaz* (MACB 100459, 100458, 99481); Saceruela, Sª de Canalizos, *A. R. Burgaz* (MACB 94144, 99419); Solana del Pino, Sª de Solana del Pino, *A. R. Burgaz et al.* (MACB 99450, 99418, 99417, 99486); Villamanrique, estribaciones Sª Morena, *A. R. Burgaz* (MACB 93542, 100446, 93543, 99488); Villarrubia de los Ojos, Sª de la Cueva, Pto. de los Santos, *A. R. Burgaz* (MACB 99485, 99483, 94145); Viso del Marques, Sª de San Andrés, Fresnedas Altas, *A. R. Burgaz* (MACB 99433); *Córdoba*: Fuente Obejuna, Valdeinfierno, *A. R. Burgaz* (MACB 93747, 93753, 99468, 100463); Villaharta, fuente de la Lastrilla, *A. R. Burgaz* (MACB 99416, 100445, 94146, 94147, 99482); Villaviciosa de Córdoba, Sª del Esparragal, río Cabrillas, *A. R. Burgaz* (MACB 100450, 100453, 100454); *Gerona*: San Martín de Ogassa, mirador de la Torre, *A. R. Burgaz* (MACB 100450); *Granada*: Alfalcar, Sª de Huetor, P. Nat. de Huetor, La Alfaguara, *A. R. Burgaz* (MACB 99459); *Guadalajara*: Gascueña de Bornoba, Sª de Alto Rey, *A. R. Burgaz* (MACB 99437, 93612); *Huelva*: Zalamea la Real, *A. R. Burgaz* (MACB 49934, 99479); *Jaén*: Barranco de Valdeazores, P. Nat. de Despeñaperros, *A. R. Burgaz* (MACB 94148); Chiclana de Segura, Embalse de Guadalmina, *A. R. Burgaz* (MACB 99487); Génave, *A. R. Burgaz* (MACB 99440); La Aliseda, *A. R. Burgaz* (MACB 99444); Montizón, cauce del río Dañador, *A. R. Burgaz* (MACB 93537, 93538, 93539); Santa Elena, *A. R. Burgaz* (MACB 100462, 99476, 93749); *Madrid*: El Cuadrón, *A. R. Burgaz* and *S. Casas* (MACB 75259, 100466); Las Matas, *A. R. Burgaz* (MACB 99438); Manzanares el Real, P. Nat. de La Pedriza, Senda de Quebrantaherraduras, *A. R. Burgaz* (MACB 100442, 94150, 100451); Monte de El Pardo, *A. R. Burgaz* (MACB 94149); Torrelaguna, *A. R. Burgaz* and *S. Casas* (MACB 75240); Torrelodones, *A. R. Burgaz* (MACB 100456); *Málaga*: Tolox, Sª de Tolox, pista en el Parque Natural, *I. Martínez* and *G. Aragón* (MACB 99490); *Salamanca*: Beleña, *A. R. Burgaz* (MACB 93610, 94151, 99460); Carpio de Azaba, *A. R. Burgaz* (MACB 100447,

99467); Frades de la Sierra, Sª de Frades, *A. R. Burgaz* (MACB 94152); Fuenterroble de Salvatierra, Sª de Frades, *A. R. Burgaz* (MACB 99470); Sancti Spiritus, *A. R. Burgaz* (MACB 99431, 99457, 99471, 99475); *Segovia*: Coca, Finca El Sequero, *A. R. Burgaz* (MACB 100441); *Sevilla*: Alanis, P. Nat. de Sª Norte, Mirador Loma del Aire, *A. R. Burgaz* (MACB 99456, 93752); Aznalcazar, *A. R. Burgaz* (MACB 99452, 99463); Cazalla de la Sierra, P. Nat. Sª Norte, Finca UPA, *A. R. Burgaz* (MACB 99462, 94359, 93754, 93751, 93750); Cazalla de la Sierra, Sª de la Grana, P. Nat. de Sª Norte, *A. R. Burgaz* (MACB 99431, 99455, 99455, 94360); La Puebla del Río, Dehesa de Abajo, *A. R. Burgaz* (MACB 99469); *Toledo*: Belvis de la Jara, *R. Pino-Bodas* (MACB 100457); Consuegra, Sª de Valdehierro, *A. R. Burgaz* (MACB 94153); El Mazo, *R. Pino-Bodas* (MACB 99477); La Iglesuela, loma de la Majada del Buey, valle del Tietar, *P. Aguilar et al.* (MACB 99445, 94361); Navaltoril, *R. Pino-Bodas* (MACB 99472, 94158); Real de San Vicente, *A. R. Burgaz* (MACB 99464); Robledo del Buey, Navalucillos, *R. Pino-Bodas* (MACB 94156); Robledo del Mazo, *R. Pino-Bodas* (MACB 94157); San Pablo de los Montes, Sª de San Pablo de los Montes, Montes de Toledo, *A. R. Burgaz* and *Mª A. Carrasco* (MACB 99447); San Roman de los Montes, Urbanización los Reguerones, *J. A. Leal and T. Pereyra* (MACB 99449, 99434); Urda, Sª Morrones, Finca El Convento, *A. R. Burgaz* (MACB 94154); Urda, subida antena de TV, *A. R. Burgaz* (MACB 94155); *Zamora*: La Tabla, *G. Aragón et al.* (MA-lichen 12655); Almaraz de Duero-Zamora, km 10, *A. R. Burgaz et al.* (MACB 70197); Canary Islands: *La Gomera*: P. Nac. Garajonay, Cumbres de Arure (Cabecera del Barranco), *C. Hdez-Padrón* and *P. L. Pérez de Paz* (MACB 93475); Parque N. Garajonay, sobre Benchijigua e Imada, *C. Hdez-Padrón and P. L. Pérez de Paz* (MACB 93474); *Tenerife*: La Esperanza, Monte de la Esperanza, pista del Acebiño, *A. R. Burgaz* (MACB 100468, 99466).

Cladonia corsicana(Rondon and Vězda) Pino-Bodas, Burgaz and M. P. Martín comb. nov.

Basionym: *Cladonia turgida* (Ehrh.) Hoffm. var. *corsicana* Rondon and Vězda in Vězda, Lich. Selecti Exsicc. Fasc. **36:** 2 (noº 881) (1970); type: France, Corsica, Dist. Ste-Marie-Sicche, Coti-Chiavari, alt. 100 m, ad terram macram graniticam in fossis viae vetustae, 5-VII-1969, *Y. Rondon et A. Vězda* [holotype, PRM, isotypes, H!, S].

MycoBank: MB 519651

Iconography in Fig. 3.

Primary thallus squamulose, persistent, squamules, 6−13 (−14) mm long x 1−3(−4) mm wide, erect, entire margin or irregularly lobate. Upper surface light green, lower surface arachnoid, white to greyish or pinkish. Podetia frequent, (4–)6−20(−24) mm long, (2–)5−10 mm wide, without scyphi, branched at the apices, entirely open, split, occasionally podetium walls are joined at the base, often with oval perforations. Podetium surface smooth, corticated, light-green. Apothecia common, dark brown. Ascospores simple (8–)9−12(−14) μm x (2.5–)3–4 μm. Pycnidia on upper surface of squamules or sometimes on podetia, globose, containing hyaline pycnidial gelatin. Conidia thread-like (7–)8−10(−12) x 0.5−1 μm.

Anatomy Squamules of primary thallus (180–)220−395 (−402) μm thick; cortex (37–)50−90 μm; algal layer (20–) 25−50 μm; medulla (105–)125−290(−305) μm. Podetial wall (140–)170−370(−400) μm thick; cortex 25−55(−63) μm; algal layer (12–)20−45(−58) μm; medulla (25–)43−138 (−175) μm; stereome (75–)80−187(−210) μm.

Chemistry Pd+red, K– or K+dark brownish, KC–, C–, UV–. Fumarprotocetraric acid and traces of protocetraric acid.

Habitat and distribution It grows on acid soils, in places with a dominant vegetation of cork oak, *Cistus* shrubs and heath-

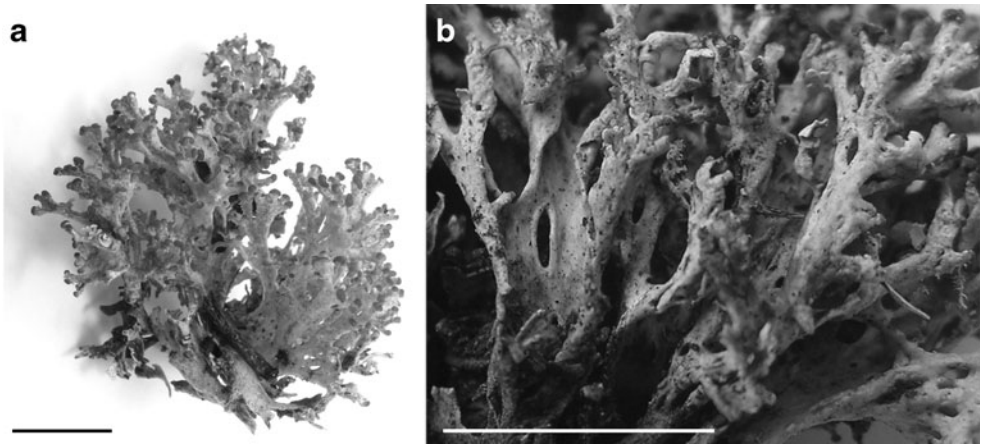

Fig. 3 *Cladonia corsicana.* **a** Thallus, **b** detail of the podetia. *Bar* 5 mm

lands, at an altitude of 55–420 m. This species grows in Corsica, Sardinia and the southwest of the Iberian Peninsula.

Remarks When podetia desiccate, they are twisting, giving the impression that their walls are joined, which makes it easy to confuse this species with *C. subturgida.*

Additional specimens examined **FRANCE:** *Corsica:* Ste-Marie-Sicche, Coti-Chiavari, *Y. Rondon and A. Vězda* (H). **ITALY:** *Sardinia:* Cagliari, Capoterra (SW of Cagliari), Santa Barbara, *F. Turno and M. C. Loi* (H). **PORTUGAL:** *Algarve:* Bensafrim, Sª do Espinhaço de Cao, *A. R. Burgaz* (MACB 101073); Sª de Monchique, zwischen Monchique und Marmelate, bei Casais, *B. Litterski* (H); *Alto Alentejo:* Pégoes, Monte das Piçarras, *A. R. Burgaz* (MACB 100764, 100762). **SPAIN:** *Cádiz:* Alcalá de los Gazules, El Picacho, Parque Natural, *A. R. Burgaz* (MACB 100765); *Córdoba:* Villaharta, fuente de la Lastrilla, *A. R. Burgaz* (MACB 100761); *Sevilla:* Cazalla de la Sierra, P. Nat. de Sª Norte, Finca UPA, *A. R. Burgaz* (MACB 100763, 101074).

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Elucidating the taxonomic rank of *Cladonia subulata* versus *C. rei* (*Cladoniaceae*)

III

ARTÍCULO III

Elucidating the taxonomic rank of *Cladonia subulata* versus *C. rei* (*Cladoniaceae*)

Raquel Pino-Bodas, Ana Rosa Burgaz & María P. Martín

Mycotaxon (2010) 113: 311-326

Cladonia subulata y *C. rei* son especies de líquenes que, desde el punto de vista morfológico, están en apariencia estrechamente relacionadas. Ambas especies presentan una morfología muy variable, siendo difícil establecer los límites entre ellas, por lo que su rango de especie se ha puesto en duda. Algunos autores destacaron que ninguno de los caracteres morfológicos utilizados hasta el momento (la coloración de los podecios, la presencia de una zona corticada en la base de los podecios, los soredios farináceos o granulosos y la presencia de escuámulas en la base de los podecios) permitía distinguir estos taxones con seguridad. Sin embargo, se han diferenciado por su diferente contenido en sustancias liquénicas. *Cladonia subulata* contiene ácido fumarprotocetrárico mientras que *C. rei* contiene ácido homosekikaico, muchas veces acompañado de ácido fumarprotocetrárico. En el presente trabajo se estudió la variación morfológica, química y anatómica de estos taxones correlacionada con datos moleculares. Se utilizaron tres regiones génicas (ITS rDNA, *rpb2* y *ef1α*), que se analizaron mediante máxima parsimonia e inferencia bayesiana. Los resultados de los análisis filogenéticos revelaron dos clados monofiléticos con alto apoyo, que se correlacionaron con los dos taxones. Los análisis morfológicos mostraron que existía una correlación entre los dos clados y los siguientes caracteres morfológicos: la presencia o ausencia de escuámulas sobre los podecios, la presencia o ausencia de córtex en la base de los podecios, el tamaño de los soredios y el grosor de la pared del podecio. Estos clados también están apoyados por diferencias anatómicas. La superficie del estereoma está reticulada, conteniendo numerosos poros, en *C. subulata,* mientras que en *C. rei* no se han observado ni poros ni

estructura reticulada. Los resultados obtenidos en este trabajo apoyan que se mantengan *C. subulata* y *C. rei* como dos especies independientes. Esta conclusión está fundada en 1) la concordancia genealógica de las tres regiones estudiadas, 2) la existencia de una correlación entre los caracteres morfológicos y los clados y 3) el hecho de que ambas especies tienen diferente hábitat.

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Elucidating the taxonomic rank of *Cladonia subulata* versus *C. rei* (*Cladoniaceae*)

Raquel Pino-Bodas[1*], Ana R. Burgaz[1] & María P. Martín[2]

rpino@bio.ucm.es

[1]*Facultad de Ciencias Biológicas, Universidad Complutense*
Antonio Novais 2, 28040 Madrid, Spain

[2] *Real Jardín Botánico, CSIC*
Plaza de Murillo 2, 28014 Madrid, Spain

Abstract — *Cladonia subulata* and *C. rei* are two lichen species apparently closely related from a morphological viewpoint. Since both species also show a high morphological variability, it has been difficult to establish the limit between them, and their taxonomic classification has often been questioned. Nevertheless, they have different lichen substance contents. The present paper aims to clarify the taxonomy of *C. subulata* and *C. rei*. Their morphological, chemical, and anatomical variation is examined and correlated with the molecular data of three gene regions (ITS rDNA, *rpb2* and *ef1α*). The results of the analyses reveal two strongly supported monophyletic clades, correlated with the two taxa. We conclude that *C. subulata* and *C. rei* should be maintained as two different species.

Key Words — *Ascomycota,* secondary chemistry, sibling species, species delimitation

Introduction

The lichens *Cladonia subulata* (L.) F.H. Wigg. and *Cladonia rei* Schaer. can be difficult to distinguish and therefore their taxonomic distinction has recently been questioned, particularly by Spier & Aptroot (2007). Traditionally, they have been regarded as two distinct species in spite of their great morphological similarity. *Cladonia subulata* is even the nomenclatural type species of the large genus *Cladonia* (Ahti 2000). The secondary metabolites, the presence of corticated areas at the base of podetia and the farinose or granular soredia are the main characters used to distinguish those species (Suominen & Ahti 1966, Wirth 1995, Brodo et al. 2001, James 2009).

Paus et al. (1993), who conducted an exhaustive revision of the morphological characters used to differentiate these species, concluded that none of them were sufficient to distinguish the two taxa. Nevertheless, they were attributed

Table 1. Specimens included in molecular study and GenBank accession numbers.

Taxon	Code	Chemical	UV	$FeCl_3$	Collection	ITS	*rpb2*	*ef1α*
C. rei	2REI	HSEK	+	+	Canada, Ontario, S L58841	FN868580	HM243200	HM243185
C. rei	3REI	HSEK	+	+	Sweden, Gästrikland, S F52894	FN868581	HM243201	HM243186
C. rei	4REI	HSEK	+	+	Norway, Oslo, BG L86605	FN868582	HM243202	HM243187
C. rei	5REI	HSEK	+	+	Canada, Newfoundland, BG L86394	FN868583	HM243203	HM243188
C. rei	6REI	HSEK	+	+	USA, Minnesota, S F53070	FN868584	HM243204	HM243189
C. rei	7REI	FUM, HSEK	+	+	Spain, Gerona, MACB 92216	FN868585	HM243205	HM243190
C. rei	8REI	FUM, HSEK	+	+	Spain, Barcelona, MACB 100473	FN868586	HM243206	HM243191
C. rei	11REI	HSEK	+	+	Slovakia, Trenčin, BRA 10005	FN868591	-	HM243192
C. rei	12REI	HSEK	-	+	Czech Republic, Central Bohemia, BRA 10044	FN868592	-	-
C. rei	15REI	FUM, HSEK	+	+	Netherlands, Utrecht, Aptroot 68588	FN868590	HM243207	HM243193
C. rei	16REI	HSEK	+	+	Japan, Akita, UPS L170710	FN868593	-	-
C. rei	17REI	FUM, HSEK	+	+	Czech Republic, Karlovy Vary, J. Vondrák 7024	FN868587	HM243208	HM243194
C. rei	18REI	FUM, HSEK	+	+	Czech Republic, South Bohemia, J. Vondrák 7006	FN868588	HM243209	HM243195
C. rei	19REI	FUM, HSEK	+	+	Czech Republic, Karlovy Vary, J. Vondrák 7026	FN868589	-	HM243196
C. subulata	1SUBU	FUM	-	-	Spain, Asturias, MACB 93151	FN868566	HM243210	HM243174
C. subulata	2SUBU	FUM	-	-	Spain, Ávila, MACB 93837	FN868567	HM243211	HM243175
C. subulata	3SUBU	FUM	-	-	Sweden, Gästrikland, S F52879	FN868568	HM243212	HM243176
C. subulata	4SUBU	FUM	-	-	Sweden, Halland, S F90966	FN868569	HM243213	HM243177
C. subulata	5SUBU	FUM	-	-	Spain, Burgos, MACB 97275	FN868570	HM243214	HM243178
C. subulata	6SUBU	FUM	-	-	Spain, Palencia, MACB 95159	FN868577	-	-
C. subulata	7SUBU	FUM	-	-	Spain, La Rioja, MACB 96350	FN868571	HM243215	HM243179
C. subulata	8SUBU	FUM	-	-	Portugal, Trás-os-Montes, MACB 93692	FN868572	HM243216	HM243180
C. subulata	9SUBU	FUM	-	-	Chile, Navarino Island, MACB 92216	FN868578	-	-
C. subulata	12SUBU	FUM	-	-	Slovakia, Moravia, BRA 10048	-	-	HM243181
C. subulata	13SUBU	FUM	-	-	Netherlands, Utrecht, L Spier	FN868573	HM243217	-
C. subulata	15SUBU	FUM	-	-	France, Midi-Pyrénées, L 75293	FN868579	-	-
C. subulata	16SUBU	FUM	-	-	Czech Republic, Central Bohemia, J. Vondrák 6983	FN868574	-	HM243182
C. subulata	18SUBU	FUM	-	-	Denmark, Zealand, J. Vondrák 6967	FN868575	-	HM243183
C. subulata	19SUBU	FUM	-	-	Austria, Upper Austria, FB	FN868576	HM243218	HM243184
C. glauca	1GLAU	SQUA			Spain, Segovia, MACB 96751	FN868594	HM243219	HM243197
C. glauca	3GLAU	BAR, THAM			Spain, Alava, MACB 96090	FN868595	HM243220	HM243198
C. cenotea	1CENO	SQUA			Denmark, Hovedstaden, J. Vondrák 6965	FN868596	HM243221	HM243199

FUM= fumarprotocetraric acid, HSEK= homosekikaic acid, SQUA= squamatic acid, BAR= barbatic acid, THAM = thamnolic acid.

a species rank based on their different habitat preferences. Spier & Aptroot (2007), on the contrary, concluded that as there are not enough characters to maintain the two taxa as independent they represent chemotypes of a single species. Syrek & Kukwa (2008) and James (2009), who have not accepted this viewpoint, retain *C. subulata* and *C. rei* as independent species.

The aim of this study is to resolve the complex *C. subulata*-*C. rei* and attempt to elucidate whether the complex represents two species or chemotypes of the one and the same species. To this end, three gene regions ITS rDNA, *rpb2* and *ef1*α have been analyzed in combination with morphological and anatomical characters. Recent studies using DNA sequence data have clarified relationships in several lichen species with high morphological similarities (Argüello et al. 2007, Ohmura & Kanda 2004, Amtoft et al. 2008).

Material & methods

Lichen material

A total of 241 specimens of *Cladonia subulata* and 60 of *C. rei* were studied. The samples selected for molecular and morphological study were chosen from several places within the geographical range of these species and are listed in Table 1. Some morphologically similar species, such as *C. glauca* Flörke and *C. cenotea* (Ach.) Schaer., were included (Suominen & Ahti 1966, Nourish 1977, Paus 1997, James 2009). *Cladonia cariosa* (Ach.) Spreng. was used as an outgroup because it was basal in the clade where *C. subulata* and *C. rei* were included by Stenroos et al. (2002) in their phylogenetic trees.

Morphological and chemical data

The samples were identified on the basis of morphology and secondary metabolites. The presence/absence of cortex at the base of podetia, presence/absence of squamules, branching type of podetia (type I: branched antler-like; type II: unbranched or forked at the apex), and cup shape of the podetia were studied macroscopically with a stereomicroscope, and the soredial size was measured under the light microscope. Microscopic measurements of the podetial wall thickness were carried out on sections cut with a freezing microtome. Iodine reactions were tested using Lugol's solution after pre-treatment with 10% KOH. In addition, transverse and lengthwise sections at the base of the podetia were made and stained with lactophenol blue solution. The stereome surface was observed by Scanning Electron Microscopy (SEM) in longitudinal sections of the podetia. Statistical analyses were done by Statgraphics 5.1 computer program. The continuous characters normality and homogeneous variance were subject to analysis of variance (ANOVA) in association with the resulting clades of the phylogenetical analyses. Continuous characters that did not fulfill the normality and homogeneous variance were analyzed by Kruskal-Wallis test. The Kolmogorov-Simirnov test was used to check normality and Levence statistic to check the homogeneous variance. Binary characters were subjected to a test of contingency tables based on χ^2 –statistic test.

Chemical composition was checked by thin layer chromatography (TLC) according to the standardized procedures of White & James (1985), with solvent systems A and

B. Moreover, 60 samples were visualized under UV light (Table 1), and $FeCl_3$ reaction (alcoholic dissolution to 10%) was checked on 188 specimens (Table 1).

DNA extraction and PCR

Total DNA was extracted using DNeasy Plant Mini Kit (Quiagen) following the manufacturer's instructions. The DNA was dissolved in 200 µl of buffer included in the kit. Three genetic regions were selected: ITS rDNA, *rpb2* partial gene, and *ef1α* partial gene. The primers used to amplify the nuclear ITS rDNA were ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990), alternatively 1780-5'F/LSU0012 (Piercey-Normore & DePriest 2001) or ITSCld /ITSClr (Pino-Bodas unpubl. data). The *rpb2* partial gene was amplified using nested PCR. The first PCR was performed with the primer pair RPB2-5F/ RPB2-7cR (Liu et al. 1999); 1 µl of the first amplification served as DNA template for a second reaction using the primers RPB2dRaq (5' GCTGCTAAGTCTACCAT 3') /RPB2rRaq (5' ATCATGCTTGGAATCTC 3') newly designed in this study. The primers used to amplify *ef1α* partial gene were CLEF-3F/CLEF-3R (Yahr et al. 2006). The amplification program for ITS rDNA was: initial denaturation at 94 ºC for 5 min; 5 cycles of 94 ºC for 30 s, 54 ºC for 30 s and 72 ºC for 1 min; and 33 cycles of 94 ºC for 30 s, 48 ºC for 30 s and 72 ºC for 1 min; with a final extension at 72 ºC for 10 min. The amplification program for *rpb2* was: initial denaturation at 94 ºC for 5 min; 40 cycles of 95 ºC for 30 s, 52 ºC for 30 s and 72 ºC for 2 min; with a final extension at 72 ºC for 10 min. The amplification program for *ef1*α was: initial denaturation at 94 ºC for 5 min; 35 cycles of 95 ºC for 30s, 55 ºC for 30s and 72 ºC for 1 min; with a final extension at 72 ºC for 10 min. PCRs were carried out with Ready-to-Go-PCR Beads (GE Healthcare Life Sciences, UK). Amplifications were prepared for a 25 µl final volume. PCR was performed using the MJ Reseach-PTC-200 termocycler (Massachusetts). The PCR products were purified using the QIAquick Kit (QIAGEN, Valencia, California, USA).

DNA sequencing

The primers for sequencing reactions were those used in PCR amplification. The sequencing reactions were done at the Secugen S. L. (CIB, Madrid, Spain) or Macrogen (Korea) sequencing service (www.macrogen.com). Sequencher™ program (Gene Codes Corporation, Inc, Ann Arbor, Michigan, USA) was used to assemble the consensus sequence from the two strands of each isolate.

Sequence alignments and data analysis

The sequences were manually aligned with SE-AL v2.0a11 Carbon (Rambaut 1996) with each region aligned separately. The transitions and transversions were considered for aligning the sequences. The ambiguous positions were removed.

After each gene region was separately analyzed, a matrix combining the three studied gene regions was constructed in which we included only taxa for which sequences of all three gene regions were available. Both individual regions and the combined matrix were analyzed using Maximum Parsimony (MP) and Bayesian Analysis. MP analyses were conducted with PAUP* version 4.0b10 (Swofford 2002) using heuristic search with 500 replicates and TBR Branch-swapping option. Bootstrap analyses were performed with 10.000 replicates, using the fast-step option. MrModeltest (Nylander 2004) was used for selecting the best evolution model (Table 2) for each region. Bayesian analyses were

carried out by MrBayes 3.1 (Huelsenbeck & Ronquist 2001). The posterior probabilities were approximated by sampling trees using Markov Chain Monte Carlo (MCMC). The posterior probabilities of each branch were calculated by counting the frequency of trees visited during MCMC analysis. Model parameters were estimated in each analysis for 2.000.000 generations sampled in 12 simultaneous chains and every 100th was saved into a file. Plots of likelihood were examined for each run to determine the number of generations required to reach stationarity (burn-in) by Tracer v.1.0. (http://tree.bio.ed.ac.uk/software/tracer/). Then, the MCMC convergence was evaluated by performing cumulative and sliding window analyses of posterior probability and among-run variability of cumulate and split frequencies using the online application AWTY (Nylander et al. 2008). The initial 2000 trees were discarded. Using the "sumt" command of MrBayes, the 50% majority-rule consensus tree was calculated from 36,000 trees sampled after reaching likelihood convergence to calculate the posterior probabilities of the tree nodes. The statistical congruence among the different regions was tested using ILD test (Farris et al. 1994; Huelsenbeck et al. 1996) carried out with PAUP. A conflict between ITS and *rpb2* and ITS and *ef1α* was found. The incongruities detected among the different data sets appeared in the *C. rei* clade. When incongruities appear among the different data sets, these sets can be analyzed as a whole or separately. This work followed the methodology proposed by Wiens (1998), who advises to separately analyze each data set and to assess the support of each clade; then to carry out a combined analysis of all the data sets, finally deeming as questionable those parts of the tree where incongruities are found.

Results

Phylogenetic analyses

In this work, 80 new sequences have been generated, of which 32 are of ITS rDNA, 22 of *rpb2,* and 26 of *ef1α*. The alignment of the ITS rDNA region contained 582 positions while the *rpb2* and *ef1α* alignments contained 891 and 612, respectively.

The MP analyses based on ITS rDNA region generated 500 equally parsimonious trees of 127 steps. The likelihood parameters of Bayesian analyses are shown in Table 2. Both analyses generated topologically similar trees. The majority Bayesian consensus tree (Fig. 1A) shows three strongly supported monophyletic clades. One clade groups all the specimens delimited as *C. subulata*; another clade includes all the samples identified as *C. rei*; and the third clade comprises the samples of *C. glauca* and *C. cenotea*. Within the *C. rei* clade, two strongly supported subclades appear. In both subclades, the specimens come from different geographical origins (Table 1).

The MP and Bayesian analyses based on *rpb2* partial gene display a similar topology (Fig. 1B). The MP analysis generated 500 equally parsimonious trees, 162 steps long. The rest of the parameters, together with the likelihood values of the Bayesian analysis are shown in Table 2. As in the ITS rDNA analyses, three strongly supported clades appear, one corresponding to *C. subulata*, another to *C. rei* and a third including *C. glauca* and *C. cenotea*. Only one strongly

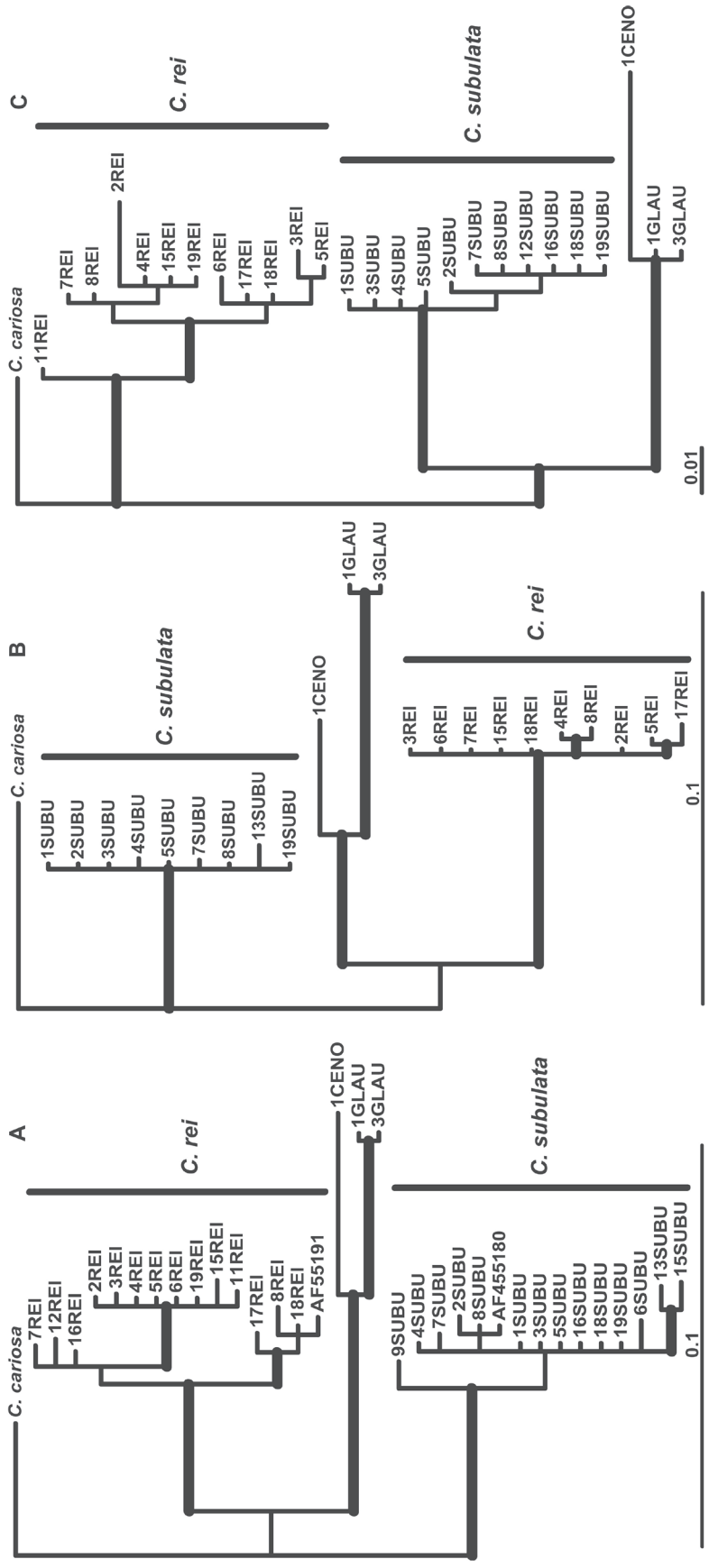

Fig. 1. Phylogeny of *Cladonia subulata* and *Cladonia rei*. The 50% consensus majority-rule tree from Bayesian/MCMC of three separate regions. Bold branches indicate a support of bootstrap ≥ al 70% and posterior probability ≥ 95%. A) ITS rnDNA B) *rpb2* C) *ef1α*.

supported subclade can be distinguished within the *C. rei* clade. However, it does not correspond to any of those appearing in the ITS rDNA analyses. The samples of this subclade have different geographical origins.

The MP analyses based on *ef1α* partial gene generated three equally parsimonious trees of 113 steps. The remaining MP parameters and Bayesian likelihood values are shown in Table 2. Analyses corresponding to this *ef1α* region also show three strongly supported monophyletic clades (Fig. 1C). In the *C. subulata* clade, one low-support subclade can be observed. The topologies of the MP and Bayesian consensus trees were not strictly identical. The MP tree shows *C. cenotea* apart from the *C. glauca* samples, while the Bayesian tree does not. The Bayesian analysis was repeated using GTR+I+G model and the result was the same.

Table 2. Information on MP analyses, evolutionary model and likelihood parameters of Bayesian analyses.

	Parameter	ITS rDNA	rpb2	ef1α	Combined
MP	CI	0.8920	0.8377	0.9292	0.8667
	RI	0.9530	0.9448	0.9815	0.9518
	RC	0.8501	0.7915	0.9120	0.8249
	informative characters	73	98	66	232
Bayesian analyses	Model	SYM+I	SYM+G	SYM+G	GTR+I+G
	-LnL	-1582.984 (0.07398)	-2128.058 (0.02592)	-1527.70 (0.00723)	-5172.63 (0.01232)
	π (A)	-	-	-	0.2601 (0.00008)
	π (C)	-	-	-	0.2519 (0.00008)
	π (G)	-	-	-	0.2415 (0.00008)
	π (T)	-	-	-	0.2465 (0.00007)
	r (A-C)	0.4745 (0.00235)	0.0468 (0.00031)	0.0605 (0.00051)	0.0591 (0.00016)
	r (A-G)	0.2498 (0.00248)	0.2375 (0.00165)	0.2485 (0.00246)	0.2472 (0.00067)
	r (A-T)	0.1474 (0.00130)	0.0873 (0.00055)	0.0808 (0.00073)	0.1150 (0.00028)
	r (C-G)	0.0666 (0.00050)	0.0326 (0.00022)	0.0312 (0.00036)	0.3326 (0.00009)
	r (C-T)	0.4095 (0.00311)	0.5272 (0.00246)	0.5026 (0.00349)	0.4944 (0.00093)
	r (G-T)	0.0566 (0.00046)	0.0684 (0.00044)	0.0760 (0.00080)	0.0608 (0.00017)
	α	-	0.2748 (0.01024)	0.3535 (0.04190)	73.254 (0.00007)
	Pinvar	0.6021 (0.00198)	-	-	0.6233 (0.00795)

Bayesian parameters: mean value (variance)
Models selected by AIC criterion using MrModeltest

The MP analyses based on the combined dataset generated 500 equally parsimonious trees of 405 steps long. The remaining parameters of the MP analyses, together with the likelihood values of the Bayesian analyses are shown in Table 2. Both analyses generated topologically similar trees (Fig. 2). Three strongly supported monophyletic clades appear, one corresponding to *C. subulata*, another to *C. rei,* and the third to *C. glauca* and *C. cenotea*.

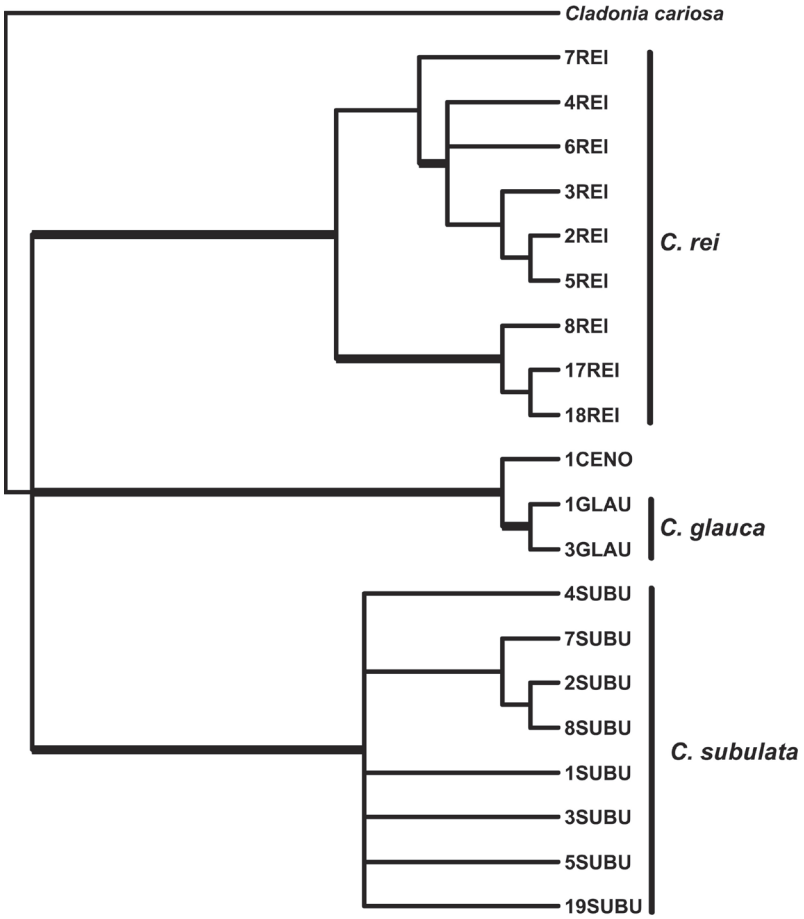

Fig. 2. The 50% consensus majority-rule tree based of combined data set (ITS rnDNA, *rpb2* partial gene and *ef1α* partial gene) from Bayesian/MCMC. The highly supported branches (bootstrap ≥ al 70% and posterior probability ≥ 95%) are indicated in bold.

The ILD-based congruence analysis revealed one conflict between the ITS rDNA + *rpb2* partial gene matrices and another conflict between the ITS rDNA + *ef1α* partial gene matrices. The cause of these incongruities lies in 4 samples of *Cladonia rei* (4REI, 8REI, 17REI and 18REI), which appear in different subclades in the analyses. The three data matrices were combined, however, in accordance with Wiens (1998).

Morphological and chemical analysis

The SEM showed notable differences between the stereome surfaces of *Cladonia subulata* and *C. rei*. In *C. rei*, the internal face of the stereome lacks pores, while *C. subulata* samples display a reticulated stereome with pores (Fig. 3). Furthermore, under the light microscope the transverse and lengthwise podetial sections (Fig. 4) reveal stereome hyphae that are thinner in *C. subulata* (2-3 μm diam.) than in *C. rei* (3.75-5 μm diam.). In both cases, the stereome hyphae are arranged lengthwise along the podetia.

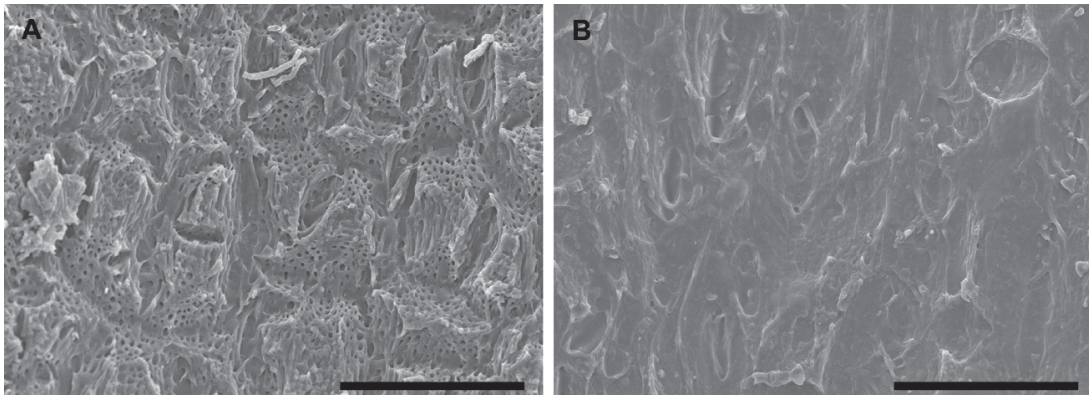

Fig. 3. SEM micrographs of the stereome surface.
A) *Cladonia subulata*. B) *C. rei*. Bar = 100 μm.

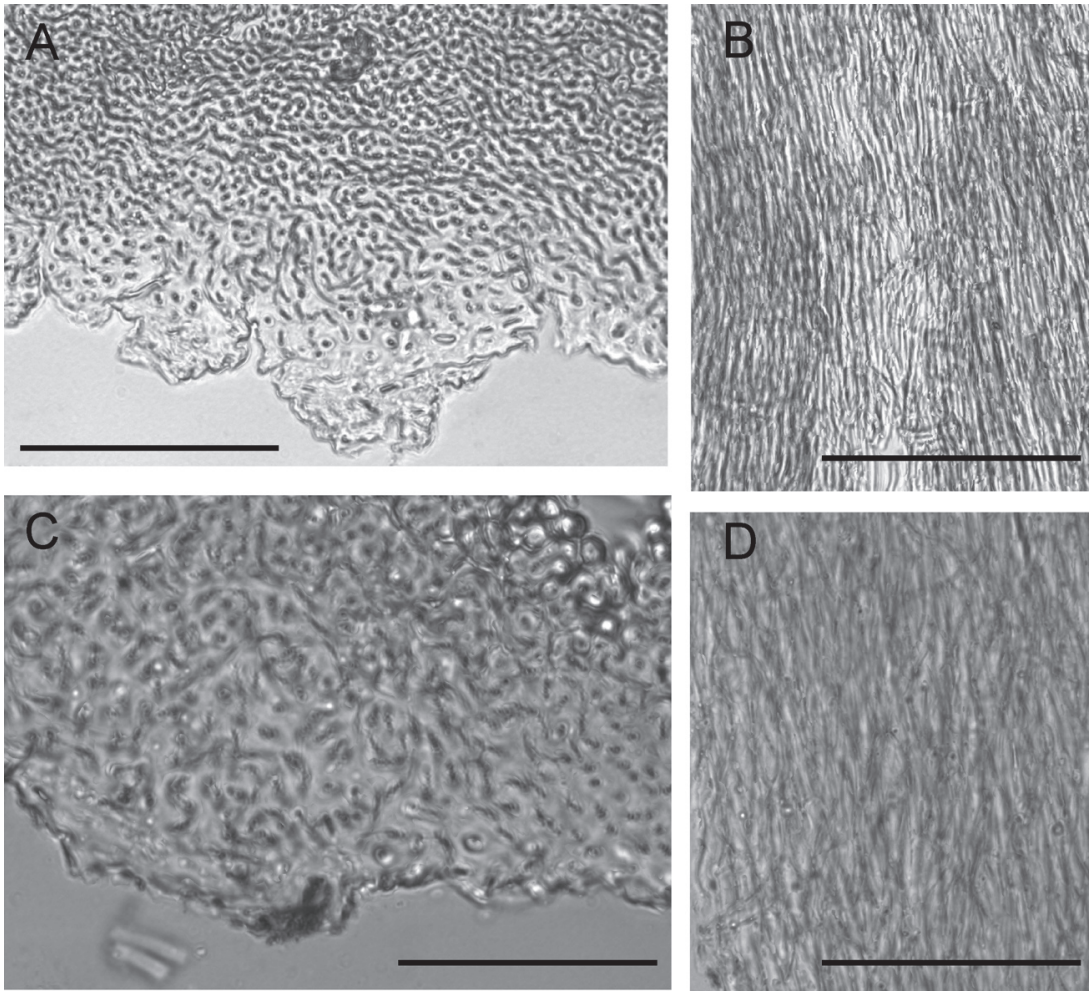

Fig. 4. Microtome sections of stereome under light microscope.
A) Transversal section of *C. subulata.* B) Lengthwise section of *C. subulata.*
C) Transversal section of *C. rei.* D) Lengthwise section of *C. rei*.
Bar = 50 μm.

Table 3. Results of the contingency table for *C. subulata* and *C. rei*.

Character	*p*
Presence/absence of basal squamules	0.035 *
Presence/absence of scyphi	0.13
Presence/absence of basal cortex	0.00008 **
Branching type I/branching type II	0.196

p, significance level (* $p < 0.05$, ** $p < 0.01$).

The contingency table (Table 3) shows the correlation between the qualitative morphological characters previously used to distinguish these taxa and the clades implied by the phylogenetic analyses. Significant differences are observed, such as the presence/absence of squamules and the presence of basal cortex on the podetia, while there are no significant differences between both taxa in the podetial branching type. Significant statistical differences were found in the podetial anatomical characters (Table 4), with the podetial wall being thicker in *C. rei* than in *C. subulata*, as also the medulla and stereome layers are, with the stereome/medulla ratio higher in *C. subulata*. Also, the soredial granules are significantly larger in *C. rei* than in *C. subulata*.

Table 4. Statistical analyses for continuous characters.

Character	*C. subulata*	*C. rei*	*p*
Soredium size	17.5-80 (125)	(14.5) 20-65 (100)	$4.42e^{-8}$**
Podetium thickness	115-310 (350)	(112.5) 130-400 (707.5)	0.0054**
Medule thickness	47.5-225 (250)	(22.5) 30-227.5 (260)	0.0029**
Stereome thickness	35-145 (187.5)	(14.5) 20-212.5 (400)	0.0000**
stereome/medule ratio	1.36-5.0 (5.70)	(1.22) 1.27-2.63 (3.08)	$6.57e^{-11}$**

The minimum value corresponds to percentile 1 and the maximum to percentile 95. The absolute maximum and minimum values are in brackets.

p, significance level (* $p < 0.05$, ** $p < 0.01$).

TLC analyses revealed that 36 samples of *C. rei* contained homosekikaic acid together with fumarprotocetraric acid, while 24 samples contained only homosekikaic acid. In both cases, homosekikaic acid was accompanied by small amounts of sekikaic acid. Furthermore, in the samples of *C. rei* the accessory substance 4'-O-methylnorhomosekikaic acid was found. Frequently fumarprotocetraric acid is accompanied by protocetraric acid; besides, in 8 of the samples containing fumarprotocetraric acid, also confumarprotocetraric acid was detected. In all *C. subulata* samples fumarprotocetraric acid was present with protocetraric acid. In addition, in 34 of these samples the satellite substance confumarprotocetraric acid occurred.

The UV test, traditionally used to detect the presence of homosekikaic acid, was applied to 60 samples; 87.5% of the samples where TLC detected homosekikaic acid gave a positive fluorescence. On the other hand, 96%

of the samples where TLC detected only fumarprotocetraric acid gave no fluorescence. The $FeCl_3$ test applied to 188 samples gave a positive reaction in 90% of the samples containing homosekikaic acid and was negative in 98% of the specimens containing only fumarprotocetraric acid.

Discussion

Evaluation of characters

Soredium size. Soredium size is one of the main characters used for species differentiation in many *Cladonia* species, as in the complex *C. chlorophaea* (Flörke ex Sommerf.) Spreng.–*C. fimbriata* (L.) Fr. (Hennings 1983). However, in *C. ochrochlora* Flörke the soredium size is variable (Hammer 1993). Statistically significant differences in soredium size were found in *C. subulata* and *C. rei,* with the soredial granules being bigger in *C. rei* (Table 4). As several factors (e.g., age, development stage, environmental conditions) probably affect soredium size (Paus et al. 1993), using this character to distinguish these species must be used with caution.

Cortex at the base of podetia. Earlier authors have discussed the utility of the podetial cortex to differentiate *C. rei* from *C. subulata.* Paus et al. (1993) and Spier & Aptroot (2007) consider it unreliable, while Syrek & Kukwa (2008) accept it as reliably diagnostic. Although a great many of the *C. rei* specimens studied were corticated, 40.62% of the *C. subulata* podetia also have corticate bases. The presence of this cortex was sometimes difficult to observe because it was covered by soredia and could be detected only by a transversal section of the podetium.

Squamules at the base of podetia. There are statistically significant differences between the *C. subulata* and *C. rei* clades related to the presence of squamules at the base of podetia (Table 4). However, as only 34.69% of *C. rei* podetia have squamules, possession of squamules cannot be used to differentiate these two species. In fact, Evans (1930) differentiated two forms of *C. nemoxyna* (Ach.) Arnold (a synonym of *C. rei*): *C. nemoxyna* f. *fibula* (Ach.) Vainio—lacking podetial squamules—and *C. nemoxyna* f. *phyllocephala* Arn.—with squamulose podetia. The presence/absence of squamules on the podetia is actually a variable character in many *Cladonia* species, e.g., *C. furcata* (Huds.) Schrad. and *C. rangiformis* Hoffm. (Burgaz & Ahti 2009).

Morphology of podetia. The presence of antler-like, irregularly branched podetia is one character attributed to *C. subulata* (Brodo 2001, Osyczka 2006, James 2009). In the material used for this paper, however, no significant differences were found between the podetia of *C. subulata* and *C. rei*. It is worth noting that much *C. subulata* material studied here was young and not well developed. Other authors (Paus et al 1993, Spier & Aptroot 2007) consider the

podetia morphology to be of little taxonomic value due to the wide variability (simple, cup-like, irregularly branched) that podetia show.

Anatomical characters. Statistically significant differences between *Cladonia subulata* and *C. rei* were found in the thickness of the podetial wall (Table 4). Nevertheless, as in soredium size, the thickness of the podetial wall and the thickness of each layer are widely variable in these two taxa, making it difficult to identify the two species based only on these characters. On the other hand, such anatomical features can be used to differentiate other similar taxa such as *C. mediterranea* P.A. Duvign. & Abbayes from *C. mitis* Sandst., *C. ciliata* Stirt. var. *ciliata* from var. *tenuis* (Flörke) Ahti (Burgaz & Martínez 2008), or the species within the *C. gracilis* (L.) Willd. group (Ahti 1980). In some cases, some taxonomic value is attributed to the stereome surface (Ahti 1980), which is different in *C. rei* and in *C. subulata*. Under the stereomicroscope, the reticulated stereome surface of *C. subulata* and the smooth stereome surface of *C. rei* can sometimes be observed. In most cases, however, a SEM is required to observe stereome surfaces, greatly limiting its utility for an everyday identification. Besides, the differing stereome hyphal thicknesses in those species may be responsible for the differences seen on the stereome surface.

Color of the podetia. The color of the podetia of *C. subulata* reportedly varies from whitish-greyish to bright green, up to brownish green, or at least with zones of brownish coloring, while in *C. rei* the podetia vary from brownish green to dirty brown (Suominen & Ahti 1966, Thomson 1968, James 2009); nevertheless color could turn out to be an ambiguous character due to the variation within either species (Paus et al. 1993, Spier & Aptroot 2007). In the present study we found that the podetia of *C. subulata* are often pale green or whitish (though some of them present brownish zones), while in *C. rei* they are green brownish.

Chemistry. Secondary metabolites were confirmed as the only reliable characters to distinguish *C. rei* and *C. subulata*. A negative *p*-phenylenediamine (Pd) reaction is still useful in diagnosing specimens as *C. rei*. But a positive reaction is not reliable (Pišút 1961, Paus et al. 1993, Spier & Aptroot 2007), because many *C. rei* samples contain fumarprotocetraric acid in addition to homosekikaic acid, although Suominen & Ahti (1966) note that the *C. rei* Pd reaction is slow, being yellow at first, while in *C. subulata* it is normally instantly red, due to different fumarprotocetraric acid concentrations. Specimens containing homosekikaic acid do appear white under UV, but our results have shown small errors occur in detecting the presence of homosekikaic acid using the UV test. Nonetheless, we find the UV test useful in differentiating the species in most cases. Homosekikaic acid can also be detected by the ferric chloride test, which produces a violet spot when it is positive (Huneck &

Yoshimura 1996). Although this reaction is not used in the keys, we consider it useful for differentiating *C. rei* from *C. subulata*, and it should be included in the identification keys.

Delimitation of the taxa

Despite the high phenotypic similarity of *C. subulata* and *C. rei*, the phylogenetic analyses of the ITS rDNA, *rpb2* and *ef1α* regions show two strongly supported monophyletic clades. These clades agree with the chemical variability of the *C. subulata-C. rei* complex. All the specimens included in the *C. rei* clade contain homosekikaic acid with fumarprotocetraric acid as a frequent accessory substance, while in the *C. subulata* clade no specimens with homosekikaic acid were found. If the taxa belonged to a single species with two (to three) chemotypes, it should be expected that the chemotypes would appear intermingled, which is not true. Besides, each clade is associated with a different set of morphological characters.

In addition, the two species have obviously different ecological requirements. *Cladonia rei* is a terricolous species growing in open areas with low humus content and subneutrophilous substrate. It may sometimes grow on impoverished soils with high heavy metal content (Hajdúk & Lisická 1999). *Cladonia subulata* grows on humus-rich acidophilous substrates and even in shady areas (Sipman 1977, Paus et al. 1993, Hammer 1995, Syrek & Kukwa 2008). However, both species do occasionally grow on wood or bare rocks (Spier & Aptroot 2007). Both taxa are broadly distributed in Europe, Asia, and North America and have also been found in Australasia. However, *C. rei* has not been reported for South America or the Antarctic, while *C. subulata* grows in Argentina and Chile. In general *C. subulata* has a wider distribution, although absent in warm areas, while *C. rei* is more common in temperate or sub-arid areas, being absent in Arctic and Antarctic zones (Ahti in litt.).

Suominen & Ahti (1966) found that the *C. rei* chemotypes usually did not appear intermingled, suggesting that the chemotypes are genetically, not environmentally, determined. But the incongruities detected among the different data sets within the *C. rei* clade shows that phylogenetic relationships within this clade are not fully resolved (Wiens 1998).

Our results support *C. subulata* and *C. rei* as two independent phylogenetic species. This conclusion is founded on: 1) the genealogic concordance of the three gene regions; 2) the existence of a correlation between clades and morphological characters; and 3) the fact that both species have different habitats. Our data corroborate the results obtained in the phylogenetic study of *Cladonia* by Stenroos et al. (2002) and Dolnik et al. (2010) where *C. subulata* and *C. rei* appear in separate clades. Spier & Aptroot (2007) pointed out that the Canadian specimen of *C. rei* (AF455191) analyzed by Stenroos et al. (2002)

possibly belongs to another taxon than the European ones. Our ITS analysis, which included this sequence, shows it grouping with the other *C. rei* samples.

Cladonia glauca is morphologically similar to *C. rei,* sharing grey brownish podetia and squamules at the podetia base (Brodo et al. 2001, Syrek & Kukwa 2008, James 2009, Burgaz & Ahti 2009). However, they contain different lichen substances representing different biosequential groups. *Cladonia glauca* has squamatic acid or (rarely) thamnolic and barbatic acids (Burgaz et al. 1999, Burgaz & Ahti 2009). In addition, *C. glauca* presents a very peculiar groove along the podetium that distinguishes it from *C. rei*, and it is fully unable to produce cups (scyphi), which occur in mature specimens of *C. rei* and *C. subulata.* Our phylogenetic analyses clearly separate *C. glauca* from *C. rei. Cladonia glauca* seems to be related to *C. cenotea* (in some areas they can be difficult to distinguish), and Stenroos et al. (2002) cite *C. cenotea* as phylogenetically related to *C. crispata* (Ach.) Flot. and *C. subsubulata* Nyl. Nevertheless, further studies including additional taxa are necessary to establish the phylogenetic relationships of *C. glauca*.

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Species delimitations in the *Cladonia cariosa* group (*Cladoniaceae*, Ascomycota)

IV

ARTÍCULO IV

Species delimitations in the *Cladonia cariosa* group (*Cladoniaceae*, Ascomycota)

Raquel Pino-Bodas, Ana Rosa Burgaz, María P. Martín & H. Thorsten Lumbsch

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Los caracteres fenotípicos han mostrado ser insuficientes para establecer los límites entre las especies en el género *Cladonia*. El presente estudio aborda la circunscripción de especies dentro del grupo de *C. cariosa* examinando numerosos especímenes de las especies que se aceptan en la actualidad, *Cladonia cariosa*, *C. symphycarpa*, *C. acuminata*, *C. subcariosa* y *C. latiloba.* Las especies de este grupo se caracterizan por tener un talo primario persistente y podecios no escifosos. En general se desarrollan sobre sustratos calcáreos. Los caracteres utilizados para distinguir *C. cariosa* de *C. symphycarpa* son el tamaño de las escuámulas del talo primario y la cantidad de fisuras que presentan los podecios. Sin embargo, la frecuente ausencia de podecios en *C. symphycarpa* complica las identificaciones, por lo que los metabolitos secundarios han sido empleados para distinguir estas dos especies. Sin embargo, ambas comparten varios quimiótipos. El objetivo de este estudio fue establecer las fronteras entre las especies de este grupo. Se emplearon métodos de reconstrucción filogenética basados en máxima verosimilitud e inferencia bayesiana, utilizando secuencias de DNA de las regiones ITS rDNA, *rpb2* y *ef1α*. Nuestros resultados muestran que el grupo de *C. cariosa* consta al menos de cuatro linajes filogenéticos. Los resultados también muestran que cada uno de estos linajes es químicamente variable, lo que restringe el valor taxonómico de las diferencias químicas en este grupo. Sin embargo, se ha encontrado una correlación entre los distintos linajes filogenéticos y ciertos rasgos anatómicos, tales como la superficie de las escuámulas y la estructura del córtex. Mediante la comparación del material tipo, concluimos que el clado A corresponde a *C. cariosa* s.s., el clado B a *C. symphycarpa* s.s, el clado C a *C. acuminata,* mientras que la identidad del

clado D queda sin resolver. Los especímenes agrupados en este último clado tienen escuámulas similares en tamaño a las de *C. symphycarpa,* pero presentan podecios muy fisurados, semejantes a los de *C. cariosa*. Este resultado confirma que, al igual que en otros grupos de líquenes, la superficie cortical observada mediante el SEM tiene valor taxonómico.

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Species delimitations in the *Cladonia cariosa* group (*Cladoniaceae*, Ascomycota)

Raquel PINO-BODAS, Ana Rosa BURGAZ, María P. MARTÍN and H. Thorsten LUMBSCH

Abstract: Phenotypic characters, either morphological or chemical, have shown to be insufficient to delimit species boundaries in the genus *Cladonia*. The present study addresses the circumscription of species within the *Cladonia cariosa* group, examining a number of specimens of the currently accepted taxa *Cladonia cariosa*, *C. symphycarpa*, *C. acuminata*, *C. subcariosa* and *C. latiloba*. We employed maximum likelihood and Bayesian methods of phylogenetic reconstructions based on DNA sequences of ITS, *rpb2* and *ef1a* regions. Our results show that the *C. cariosa* group consists of at least four phylogenetic lineages. It is also shown that each of these lineages is chemically variable, which restricts the taxonomic value of the chemical differences within the group. However, anatomical differences, such as squamule surface and cortex structure, were found to correlate with the distinct lineages found in the phylogenetic analysis. This result confirms the taxonomic value of the cortical surface under SEM, as was found in other lichen groups.

Key words: chemistry, *Lecanorales*, lichens, taxonomy

Introduction

The circumscription of species in lichen-forming fungi has largely been based on morphological or chemical characters. Especially in groups of foliose and fruticose lichens, characters of the vegetative thallus have been widely used in the distinction of taxa. However, there is a growing body of evidence from DNA-based studies that morphological and chemical characters do not reflect the real number of species in lichenized fungi (Crespo & Lumbsch 2010). *Cladoniaceae* is a perfect example to illustrate the difficulties of species circumscriptions using phenotypic characters. Several phylogenetic studies have demonstrated a remarkable amount of phenotypic disparity in this family (Stenroos & DePriest 1998; Wedin *et al.* 2000; Stenroos *et al.* 2002*a*, *b*; Zhou *et al.* 2006; Lumbsch *et al.* 2010; Parnmen *et al.* 2010). In fact, it is well known among lichenologists that species recognition within the core genus of the family, *Cladonia*, is not an easy task (Thomson 1968; Ahti & Sohrabi 2006; Syrek & Kukwa 2008). Morphology-based species circumscriptions in *Cladonia* rely heavily on the secondary thallus, the so-called podetia, while the primary thallus is simpler and has been used to distinguish taxa only in a few instances (Ahti 2000). However, numerous species of *Cladonia* are morphologically variable (Ahti 2000; Kotelko & Piercey-Normore 2010), for example *C. furcata* (Huds.) Schrad. (Ahti & Hammer 2002), *C. ramulosa* (With.) J. R. Laundon (Ahti 2000; Burgaz & Ahti 2009) or *C. squamosa* Hoffm. (James 2009). The situation is complicated by chemical variability that is often not clearly associated with morphological differentiation.

Phylogenetic studies employing molecular data to address species circumscription in

R. Pino-Bodas and A. R. Burgaz: Departamento Biología Vegetal 1, Facultad de Biología, Universidad Complutense de Madrid, E-28040 Madrid, Spain. Email: rpino@bio.ucm.es
M. P. Martín: Departamento de Micología, Real Jardín Botánico, CSIC, E-28014 Madrid, Spain.
H. T. Lumbsch: Department of Botany, The Field Museum, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA.

Cladonia have helped to elucidate evolutionary relationships, and to determine the taxonomic relevance of the phenotypic characters in species delimitation. For instance, in the *C. arbuscula* group, a number of taxa had been described on the basis of different morphologies. However, molecular data suggest that these morphotypes belong to one morphologically variable lineage (Piercey-Normore 2010). The chemical variability in the *C. chlorophaea* group could not be correlated with PCR-RFLP patterns found in studies which used ribosomal nuclear DNA (DePriest 1993, 1994). In the *C. pyxidata* and *C. pocillum* groups, neither species was found to be monophyletic, and the morphological variation was found to be determined by soil pH (Kotelko & Piercey-Normore 2010). In the cases of *C. subulata* (L.) F. H. Wigg. and *C. rei* Schaer., two morphologically and chemically variable species whose delimitation was disputed (Spier & Aptroot 2007), the presence of different secondary metabolites was shown to be correlated with different clades identified in phylogenetic studies using molecular data (Dolnik *et al.* 2010; Pino-Bodas *et al.* 2010).

This study focuses on the *Cladonia cariosa* group, defined by Culberson (1969), Harris (1975) and Culberson *et al.* (1993) as consisting of *C. brevis* (Sandst.) Sandst., *C. cariosa* (Ach.) Spreng., *C. polycarpia* G. Merr., *C. polycarpoides* Nyl., *C. sobolescens* Nyl. ex Vain., *C. subcariosa* Nyl., *C. subclavulifera* Asahina and *C. symphycarpa* (Flörke) Fr. These species are characterized by a persistent primary thallus and ascyphose podetia. Nevertheless *Cladonia brevis*, *C. polycarpia*, *C. polycarpoides*, *C. subcariosa*, *C. sobolescens* and *C. subclavulifera* differ in their secondary metabolites (Evans 1944; Culberson 1969; Park 1985; Huovinen *et al.* 1989; Culberson *et al.* 1993), but not in morphology, and so Ahti (2000) combined them in a single species, namely *C. subcariosa*. Consequently, the *Cladonia cariosa* group then consisted of *C. cariosa*, *C. subcariosa* and *C. symphycarpa*. *Cladonia subcariosa* was later shown to be distantly related to the *C. cariosa* group despite their morphological similarity (Kärkkäinen 2000). However, *C. cariosa* and *C. symphycarpa* constitute a monophyletic group along with *C. acuminata* (Ach.) Norrl. (Stenroos *et al.* 2002*a*). The latter differs morphologically from *C. cariosa* and *C. symphycarpa* in having sorediate podetia, unbranched or dichotomously branched near the tips (Ahti 2000). Hence, the *Cladonia cariosa* group currently includes *C. acuminata*, *C. cariosa* and *C. symphycarpa*, which are the focus of this study. These three taxa have production of the secondary metabolite atranorin and a calcareous substratum in common (Stenroos 2002*a*). The morphological characters used to distinguish *C. cariosa* from *C. symphycarpa* are subtle and variable and include squamule size and the amount of podetium fissures, which are more abundant in *C. cariosa* (Stenroos *et al.* 1992; Piercey-Normore 2003; Burgaz & Ahti 2009). Species identification is further complicated by the frequent lack of podetia in *C. symphycarpa* (Masselink & Sipman 1985; Carlin & Larsson 1994). The chemical variation has been widely employed to distinguish *C. cariosa* from *C. symphycarpa*, but it was found that they share several chemotypes (Harris 1975; Culberson *et al.* 1993; Piercey-Normore 2003; Bültmann & Lünterbusch 2008; Burgaz & Ahti 2009). *Cladonia cariosa* has seven chemotypes: atranorin only (the most common chemotype, including the type material); atranorin and fumarprotocetraric acid; atranorin and homosekikaic acid; atranorin and norstictic acid; atranorin and psoromic acid; atranorin and rangiformic acid or atranorin, fumarprotocetraric and rangiformic acids. Five chemotypes have been described for *C. symphycarpa*: atranorin only; atranorin and norstictic acid (the most common chemotype); atranorin, norstictic and stictic acids; atranorin and psoromic acid; and atranorin and fumarprotocetraric acid. The chemotype containing psoromic acid was described as *C. dahliana* (Kristinsson 1974), but some authors considered it as a synonym of *C. symphycarpa*, since they are morphologically indistinguishable (Ahti 1976; Randlane 1986; Ahti & Hammer 2002; Burgaz & Ahti 2009). The chemotype with stictic acid has been found only in Tierra de Fuego

(Stenroos & Ahti 1990; Stenroos *et al.* 1992). In specimens related to *C. symphycarpa* from Iceland and Andorra, bourgeanic acid was found (Culberson *et al.* 1993; Azuaga *et al.* 2001), but the identity of this material has not been confirmed. Given the high chemical variability of *C. cariosa* and *C. symphycarpa*, and the fact that several chemotypes are present in both species, the secondary metabolites cannot be used as discriminant characters to tell them apart. *Cladonia acuminata* consists of three chemotypes that are morphologically indistinguishable (Huovinen *et al.* 1989): atranorin and norstictic acid; atranorin only; and atranorin and psoromic acid. The chemotype with psoromic acid was described as C. *norrlinii* Vain. or *C. acuminata* var. *norrlinii* Lynge Ahti (2000) showed that the type material of *C. acuminata* var. *norrlinii* contains norstictic and not psoromic acid and consequently included this taxon in *C. acuminata*. In contrast, Harris (2009) described *C. acuminans* R. C. Harris as a different species from the psoromic acid chemotype, arguing that the distribution area of two chemical variants is different. Though both chemotypes coexist in North America, the one with psoromic acid is not present in Europe.

The aim of this study is to elucidate the species boundaries within the *C. cariosa* group and to examine whether the chemically variable taxa *C. acuminata, C. cariosa* and *C. symphycarpa* are correlated with phylogenetic lineages.

Materials and Methods

Taxon sampling

In this study we checked the identifications of 323 specimens of the following species: *Cladonia cariosa* (114 samples), *C. symphycarpa* (140 samples), *C. acuminata* (19 samples), *C. subcariosa* (49 samples) and *C. latiloba* Ahti & Marcelli (1 sample). The specimens are held in the herbaria B, BG, H, L, MACB, S and UPS, including the types of *C. cariosa* (H-ACH-1577) and *C. symphycarpa* (UPS). For the molecular study, specimens were chosen from different geographical origins, including most of the known chemical variability in the group (Table 1). The species were identified using morphological characters (squamule size and morphology of podetia), according to Ahti (2000), Ahti & Hammer (2002) and Burgaz & Ahti (2009). *Cladonia subcariosa* and *C. latiloba* were used here as outgroup, based on Kärkkäinen (2000) and on our data (Appendix 1). Though they do not belong to the *C. cariosa* group, *Cladonia cariosa* and *C. latiloba* are basal to it.

DNA extraction, PCR amplification and DNA sequencing

Before DNA extraction, the secondary metabolites were extracted by soaking the specimens in acetone for two hours, and the liquid was then used for thin-layer chromatography (TLC). The DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) was used to extract DNA, according to the manufacturer's instructions. The DNA was dissolved in 200 µl of buffer included in the kit. The three following loci were amplified: nuclear ITS rDNA using primer ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990), *rpb2* using two pairs of primers, RPB2-5F/RPB2-7R (Liu *et al.* 1999) and RPB2dRaq/RPB2rRaq (Pino-Bodas *et al.* 2010), and *ef1a* using CLEF-3F/CLEF-3R (Yahr *et al.* 2006). PCRs were carried out with Ready-to-Go-PCR Beads (GE Healthcare Life Sciences, UK). The volume of reaction was 25 µl for each tube, with 0·4 mM final concentration of primers. The volume of extracted DNA used for the PCR was 1 µl. The amplification programs were: 1) 94°C for 5 min; 5 cycles of 94°C for 30 s, 54°C for 30 s and 72 °C for 1 min; and 33 cycles of 94°C for 30 s, 48°C for 30 s and 72°C for 1 min; with a final extension of 72°C for 10 min (Martín & Winka 2000) for nuclear ITS rDNA, 2) initial denaturation at 94°C for 5 min; 40 cycles of 95°C for 1 min, 52°C for 30 s and 72°C for 2 min; with a final extension at 72°C for 10 min for *rpb2* region, and 3) initial denaturation at 94°C for 5 min; 35 cycles of 95°C for 1 min, 55°C for 30 s and 72°C for 1 min; with a final extension at 72°C for 10 min for *ef1a* region. PCR products were purified using the QIAquick gel extraction Kit (QIAGEN, Hilden, Germany). The purified DNA was dissolved in 40 µl of buffer included in the kit. The sequencing reactions were done at Secugen S. L. (CIB, Madrid, Spain) and Macrogen (South Korea) service (www.macrogen.com), with the same primers used for the PCR.

Sequence alignment and phylogenetic analysis

The alignments were made manually with SE-AL v2.0a11 (Rambaut 1996) for each locus separately. Eight ambiguous positions in the ITS rDNA matrix were removed, while the matrices of *ef1a* and *rpb2* did not contain ambiguous positions. Each region was analyzed by maximum parsimony (MP) and maximum likelihood (ML). MP analyses were made using PAUP version 4.0.b.10 (Swofford 2002), using heuristic searches with 1000 random taxon-addition replicates with TBR branch swapping and MulTrees option in effect, equally weighted characters and gaps treated as missing data. For the confidence analysis the bootstrap was applied, with 10 000 replicates, using the fast-step option. Congruence among the different topologies

TABLE 1. *List of taxa and specimens, locality, collection and GenBank accession numbers used in this study*

	Taxon	Locality and collection	ITS	*rpb2*	*ef1α*
Clade A	*C. cariosa* 1	Spain, Teruel, MACB 45292	JN621908	JN621940	JN621972
	C. cariosa 2	Spain, Lérida, MACB 94208	JN621909	JN621941	JN621973
	C. cariosa 3	USA, Michigan, S F53032	JN621912	JN621944	JN621976
	C. cariosa 4	Norway, Nord-Trondelag, BG L79658	JN621913	JN621945	JN621977
	C. cariosa 5	Finland, Uusimaa, H	JN621915	JN621947	JN621905
	C. cariosa 6	Finland, Tavastia Proper, H	JN621916	JN621948	JN621979
	C. cariosa 7	Russia, Karelia Republic, H	JN621917	JN621949	JN621980
	C. cariosa 8	Canada, Manitoba, H	JN621934	JN621950	JN621981
	C. cariosa 9	Spain, Barcelona, MACB 94207	JN621907	JN621939	JN621971
Clade B	*C. symphycarpa* 1	Spain, Burgos, MACB 93496	JN621918	JN621951	JN621982
	C. symphycarpa 2	Spain, Guadalajara, MACB 93559	JN621919	JN621952	JN621983
	C. symphycarpa 3	Sweden, Öland, S L50055	JN621923	JN621956	JN621988
	C. symphycarpa 4	USA, Michigan, S F53075	JN621924	JN621957	JN621989
	C. symphycarpa 5	Germany, Oldenburg, B 60 0122320	JN621925	JN621958	JN621990
	C. symphycarpa 6	Germany, Oldenburg, B 60 0125267	JN621926	JN621959	JN621984
	C. symphycarpa 7	Bosnia and Herzegovina, Sarajevo, MACB 101124	JN621931	JN621964	JN621995
	C. symphycarpa 8	Norway, Nordland, BG L784035	JN621914	JN621946	JN621978
Clade C	*C. acuminata* 1	USA, Alaska, H	JN621932	JN621965	JN621996
	C. acuminata 2	Canada, Manitoba, H	JN621933	JN621966	JN621997
	C. acuminata 3	Chile, Región XII Magallanes y Antartida, MACB 92017	JN621920	JN621953	JN621985
	C. acuminata 4	Spain, Palencia, MACB 92739	JN621922	JN621955	JN621987
	C. acuminata 5	Canada, Manitoba, H	JN621928	JN621961	JN621992
Clade D	*C. cariosa* s. lat. 1	Spain, Gerona, MACB 94205	FR695863	HQ340075	JN621904
	C. cariosa s. lat. 2	Portugal, Tras-os-Montes, MACB 93984	JN621906	JN621938	JN621970
	C. cariosa s. lat. 3	Spain, Ávila, MACB 93018	JN621910	JN621942	JN621974
	C. cariosa s. lat. 4	Spain, Granada, MACB 92995	JN621911	JN621943	JN621975
	C. symphycarpa s. lat. 1	Spain, Madrid, MACB 92737	JN621921	JN621954	JN621986
	C. symphycarpa s. lat. 2	Austria, Steiermark, UPS L135579	JN621927	JN621960	JN621991
	C. symphycarpa s. lat. 3	Ukranie, Donts'k Oblast, H	JN621930	JN621963	JN621994
	C. symphycarpa s. lat. 4.	Russia, Tuva Republic, H	JN621929	JN621962	JN621993
Outgroup	*C. subcariosa*	USA, New Jersey, H	JN621936	JN621968	JN621999
	C. subcariosa	USA, North Carolina, H	JN621935	JN621969	JN622000
	C. latiloba	Brazil, Santa Catalina, H	JN621937	JN621967	JN621998

inferred from the loci was tested following Lutzoni *et al.* (2004). Each clade with more than 75% bootstrap support in the single-gene analyses was scanned for conflict among loci. Since no incongruence was detected among loci, the datasets were combined. MrModeltest (Nylander 2004) was used for selecting the most appropriate nucleotide substitution model for each locus using the AIC criterion. The combined dataset was analyzed by ML and a Bayesian approach. The ML analysis was implemented using Tree-Puzzle 5.2 (Schmidt *et al.* 2002) assuming a GTR+I+G model. The Bayesian analysis was carried out using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). The combined dataset was partitioned into seven sections: ITS rDNA, and each of three codon positions of *ef1α* and *rpb2*, respectively. The model SYM+G was applied to the ITS part and each partition of *ef1α*, while the K80+G model was used for all partitions of *rpb2*. The posterior probabilities were approximated by sampling trees using Markov Chain Monte Carlo (MCMC). The posterior probabilities of each branch were calculated by counting the frequency of trees visited during MCMC analysis. Two simultaneous runs with 20 000 000 generations, each starting with a random tree and employing 4 simultaneous chains, were executed. Every 1000th tree was saved into a file. The first 1 000 000 generations (i.e. the first 1000 trees) were deleted as the 'burn in' of the chain. AWTY (Nylander *et al.* 2008) was used to determine when the chains reached the stationary stage. The 50% majority-rule consensus tree was calculated using the 'sumt' command of MrBayes.

The pairwise fixation index F_{ST} (Weir & Cockerham 1984) was calculated with DnaSP v. 5 (Librado & Rozas 2009) using the combined dataset. The F_{ST} was

employed to estimate the differentiation among the clades following O'Brien *et al.* (2009) and Leavitt *et al.* (2011).

Morphology and chemistry

Microscopic measurements of the squamule thickness were carried out using hand-cut transverse sections. Three squamules of the primary thallus were measured for each specimen included in the molecular analyses (except when the material was scarce, in which case only one or two squamules were measured). The podetial squamules of *C. acuminata* were not studied. In addition, transverse sections of the squamules, cut with a Micron-ACP freezing microtome and stained with lactophenol blue solution, were made to study the cortex structure. The surfaces of the squamules were observed by Scanning Electron Microscopy (SEM) using a Hitachi S-3000N, and the specimens were prepared according to Hale (1973), vacuum-coated with gold-paladium and without critical-point drying. The statistical analyses of length, breadth, incision and thickness of the squamules, thickness of the cortex, the algal layer and the medulla were carried out with the STATGRAPHICS 5.1 software program. The Kolmogorov-Smirnov test was used to check normality, and Levene's test for equality of variances. All variables were normal and had homogeneous variances, hence we used one-way ANOVA to analyze the association of characters among the clades found in phylogenetic analyses, according to Wirth *et al.* (2008), Murillo *et al.* (2009) and Rivas-Plata *et al.* (2011). The probability level for significance was set at $P < 0{\cdot}05$. A Tukey HSD post-hoc test was performed to identify which differences among clades were significant.

The chemical composition was studied by TLC in 132 specimens following White & James (1985), using solvent systems A and B. Specimens studied by other researchers using TLC were not rechecked (unless they were used for the molecular study). Some old material not suitable for molecular studies was also not checked.

Results

Phylogenetic analyses

A total number of 97 new sequences was generated (Table 1) for this study (32 ITS rDNA, 32 *rpb2* and 33 *ef1α* sequences). The combined data matrix contained 2154 characters (628 in the ITS rDNA, 628 in the *ef1α* and 898 in the *rpb2* dataset), 1817 of which were constant, and 225 parsimony-informative (76 in the ITS rDNA, 54 in the *ef1α* and 95 in *rpb2* dataset). MP analysis generated 24 equally parsimonious trees, 490 steps long, with CI = 0·747 and RI = 0·893. ML analysis yielded a tree with a likelihood value of LnL = −6393·86, while the mean likelihood of the Bayesian tree sampling was LnL = −6108·97.

The phylogenetic reconstructions of the combined dataset using MP, ML and Bayesian analyses yielded trees with similar topologies. Figure 1 shows the tree of the Bayesian analysis. The specimens of the *C. cariosa* group form a strongly supported monophyletic group in all analyses. Clade A gathers only *C. cariosa* s.str. specimens, and clade B includes only *C. symphycarpa* specimens. *Cladonia acuminata* specimens (clade C) form a monophyletic group. A fourth clade (clade D) includes specimens that were identified as *C. cariosa* or *C. symphycarpa* on the basis of morphology and chemistry. The pairwise F_{ST} values showed genetic differentiation among the clades. The values varied from 0·59 to 0·79 (Table 2).

Morphological and chemical results

A re-examination of morphological characters revealed differences among the clades found in the phylogenetic analysis of the DNA sequence data. Clade A includes specimens with squamules significantly shorter than those in clades B and D and thinner than in clade B (Tables 3, 4). These squamules can be entire or have incisions that reach up to 30% of the entire squamule length. When observed using SEM, the surface of the squamules is smooth in some specimens, while in others it shows fissures and small cells that commonly do not exceed 10 μm in diameter (Fig. 2A). In transverse sections the cortex is smooth, and two layers can be distinguished within the upper cortex (Fig 2B). The outer cortex does not stain in lactophenol cotton-blue, indicating that this layer consists of dead mycobiont hyphal cells (i.e. an epinecral layer). Specimens in clade B usually have cracked squamules, which are significantly longer and thicker than those of clade A (Table 4). Their surface appears nearly smooth in SEM images, but with wide, shallow fissures (Fig. 2C). The cortex in clade B is thick and homogeneous and lacks an epinecral layer (Fig. 2D). Clade C includes specimens with squamules similar

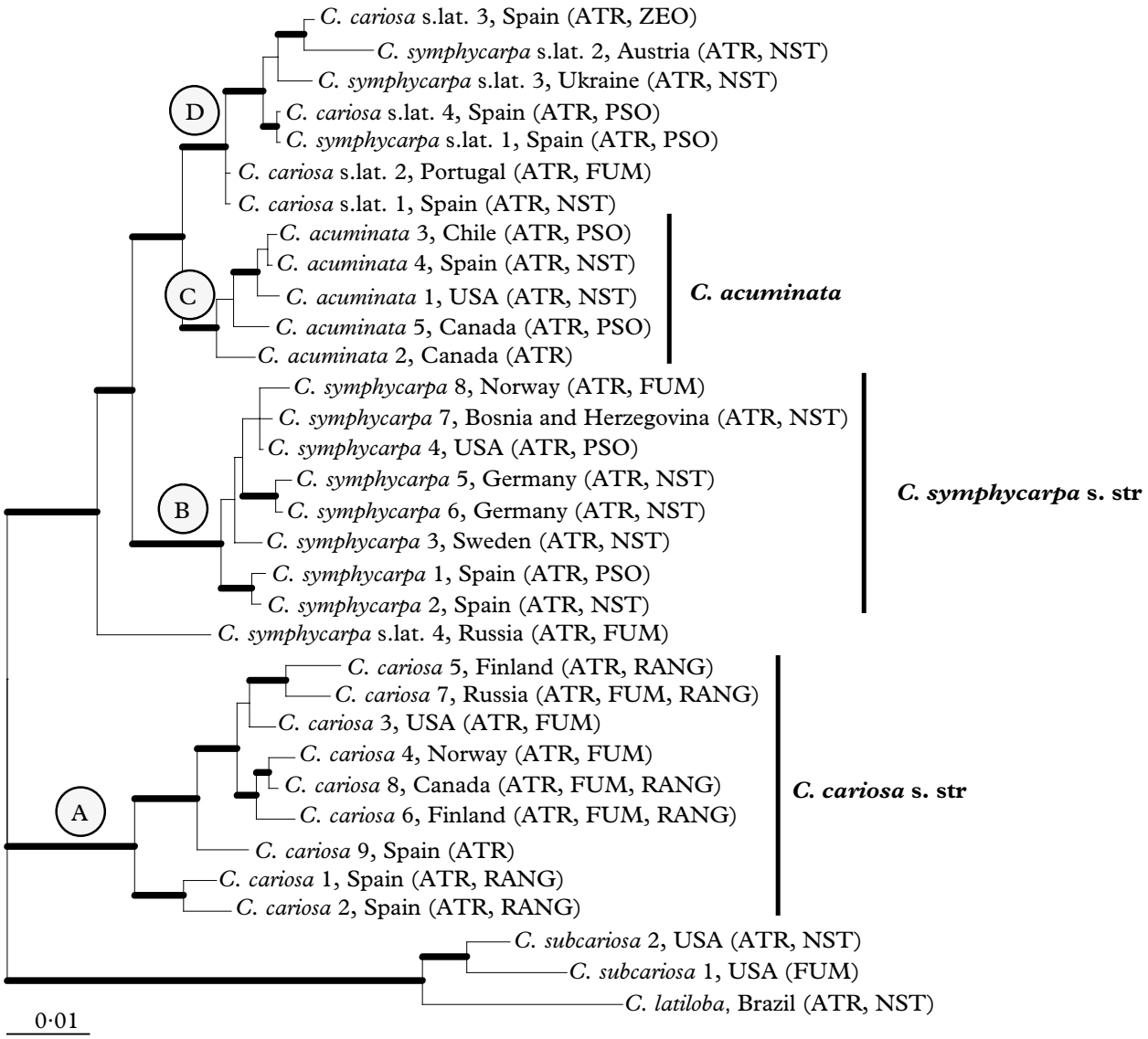


FIG. 1. Phylogeny of the *Cladonia cariosa* group based on a combined dataset (ITS rDNA, *rpb2* and *ef1a*). This is a 50% majority rule consensus tree of a Bayesian analysis. Branches supported with posterior probability $\geq$ 0·95 and bootstrap > 70 % are indicated in bold. ATR = atranorin, FUM = fumarprotocetraric acid, NST = norstictic acid, PSO = psoromic acid, RANG = rangiformic acid.

in size to those of clade B. The surface of the squamules is rough, with shallow cracks, showing an areolate-verrucose surface (Fig. 2E), and the cortex is homogeneous, without an epinecral layer (Fig. 2F). Squamules in clade D are similar in size to those in clades B and C. The squamule surfaces are strongly fissured, usually showing small cells about 5 µm in diameter, similar to those in clade A (Fig. 2G). Transverse sections of the cortex show a similar anatomy to those in clades B and C, lacking a well differentiated epinecral layer (Fig. 2H).

TABLE 2. *Pairwise F_{ST} values for combined dataset among clades*

	F_{ST}
Clade A-B	0·79823
Clade A-C	0·79425
Clade A-D	0·78799
Clade B-C	0·78610
Clade B-D	0·79260
Clade C-D	0·59728

TABLE 3. *Variation (mean ± standard deviation) of morphological and anatomical characters of squamules in comparison with phylogenetic pattern*

	Clade A $n = 20$	Clade B $n = 24$	Clade C $n = 12$	Clade D $n = 20$	*P*
Length (mm)	2·51 ± 1·136	5·97 ± 2·397	5·04 ± 2·685	5·81 ± 1·419	0·002*
Width (mm)	1·19 ± 0·235	1·91 ± 0·653	2·22 ± 0·824	1·99 ± 0·727	0·03 *
Incision/length (mm)	0·12 ± 0·147	0·36 ± 0·201	0·23 ± 0·189	0·32 ± 0·259	0·06
Thickness (μm)	236·56 ± 65·771	340·09 ± 75·180	318·59 ± 64·452	275·84 ± 28·748	0·008*
Cortex (μm)	45·31 ± 10·134	62·93 ± 9·999	62·37 ± 9·265	60·10 ± 9·811	0·002*
Algal layer (μm)	29·71 ± 4·044	35·43 ± 6·348	37·22 ± 8·656	34·33 ± 4·210	0·091
Medulla (μm)	161·55 ± 58·114	241·73 ± 72·639	219 ± 57·968	182·63 ± 21·878	0·031*

* significant *P* values (< 0·05).

TABLE 4. *Tukey's multiple comparison test for significant results of the ANOVA analyses*

	Length	Width	Thickness	Cortex	Medulla
Clade A-B	$P < 0·05$	ns	$P < 0.05$	$P < 0·05$	$P < 0·05$
Clade A-C	ns	$P < 0·05$	ns	$P < 0·05$	ns
Clade A-D	$P < 0·05$	ns	ns	$P < 0·05$	ns
Clade B-C	ns	ns	ns	ns	ns
Clade B-D	ns	ns	ns	ns	ns
Clade C-D	ns	ns	ns	ns	ns

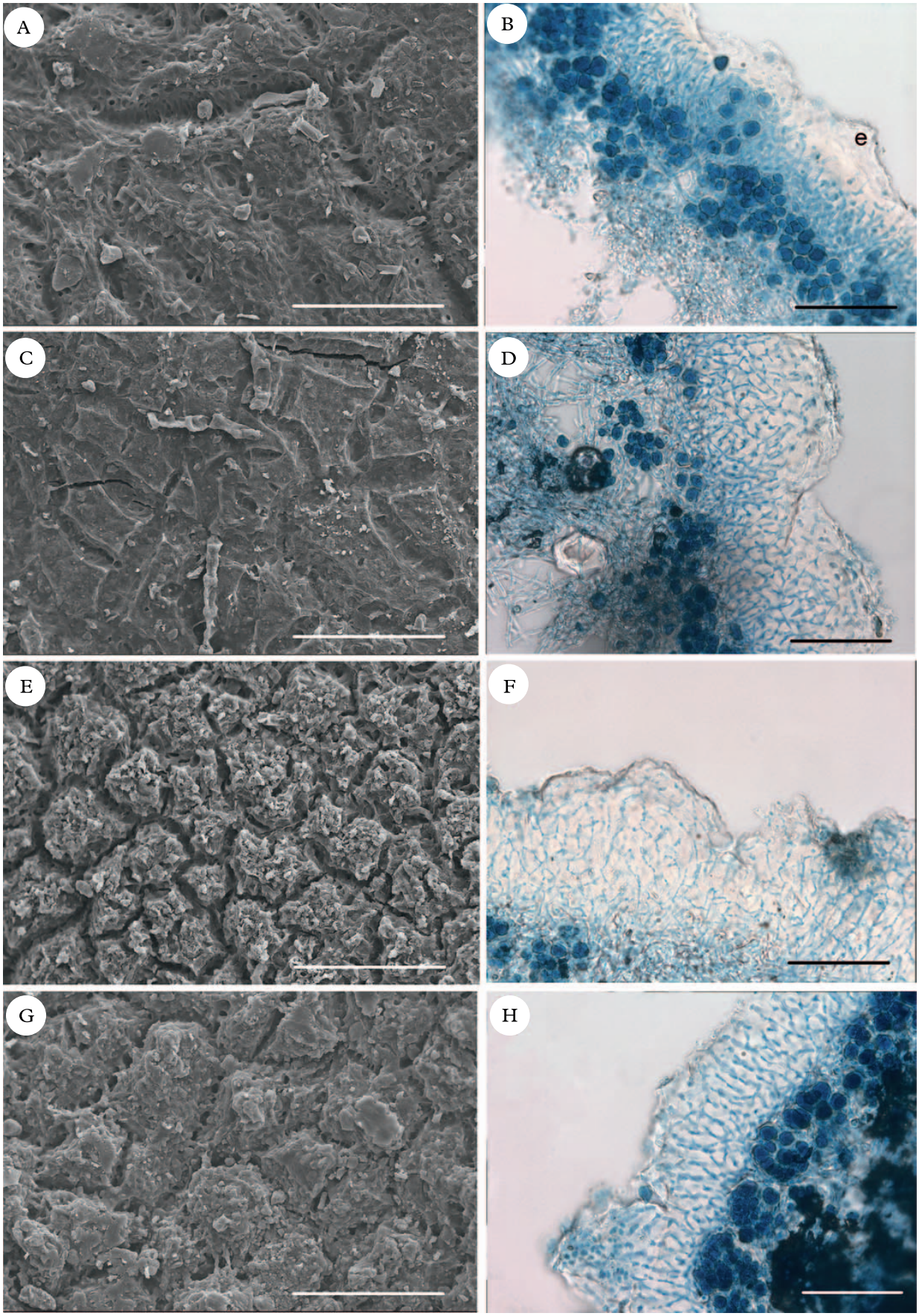
ns = not significant.

Table 5 summarizes the chemical results. The most frequent chemotype in *C. cariosa* is the one which contains atranorin alone, present in 18 specimens. In *C. symphycarpa* the most common is the chemotype with atranorin and norstictic acid. This chemotype is also the most frequent in *C. acuminata*. The chemotype of *C. cariosa* containing atranorin and homosekikaic acid, and the chemotypes of *C. symphycarpa* containing stictic acid and bourgeanic acid were not found among the specimens examined. The distribution of the different chemotypes in the clades, resulting from phylogenetic analyses, is shown in Figure 1 (which also shows the secondary metabolites found in the outgroup). Each clade included samples of several chemotypes.

Discussion

The diversity in this group falls into four strongly supported monophyletic lineages. The results of the F_{st} value estimations show low gene flow among these clades and support the separation of the distinct clades in the group. The use of F_{st} values to assess the gene flow and genetic differentiation, and to test species boundaries, follows Porter (1990). This author's method, known as Hybrid Zone Barrier, is especially useful in the recognition of species that have recently diverged (Shaffer & Thomson 2007). Subsequently, the F_{st} value has been applied to species delimitation in different organisms (Milankov *et al.* 2008; Korczynska *et al.* 2010; Mendonça *et al.* 2011), including lichenized fungi (O'Brien *et al.* 2009; Leavitt *et al.* 2011).

Following a genealogical concordance phylogenetic species recognition concept (Taylor *et al.* 2000), four species should be distinguished in the *C. cariosa* group instead of the three currently accepted. These four lineages are also supported by subtle morphological differences, such as squamule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size, thickness and surface of the squamules, and the cortex structure. These characters had previously been proposed by some authors to delimit the species within this group. The length, breadth and thickness of the primary squamules were used by Thomson (1983) and Ahti (2000) to distinguish *C. cariosa* from *C. symphycarpa*, and by Merrill (1909) to distinguish *C. subcariosa* from *C. symphycarpa*. Ahti (2000) and Ahti & Hammer (2002) noticed that squamule surfaces of *C. symphycarpa* were papillose-maculate when squamules reached maturity. Based on these preliminary observations, we were encouraged to study the squamule surfaces in more detail. The SEM observations show the existence of some micromorphological differences in the cortical surface of the different phylogenetic lineages that were previously unnoticed. The taxonomic value of the cortex surface was noted in *Alectoria* and *Cornicularia* (Hawksworth 1969) and in other *Parmeliaceae* (Hale 1973). In contrast to subtle morphological characters, our study clearly demonstrates that presence or absence of secondary metabolites is of limited taxonomic value in the *C. cariosa* group, as previously suggested (Bültmann & Lünterbusch 2008; Burgaz & Ahti 2009). A number of lineages include different chemotypes, and these chemotypes are largely shared among lineages. This is consistent with studies in some other groups of lichenized fungi, such as *Bryoria* in which the sections did not correlate with chemical characters (Myllys *et al.* 2011) or *Xanthoparmelia*, in which lineages consisted of different chemotypes (Leavitt *et al.* 2011).

Below, we attempt to clarify the identity of the clades found in our phylogenetic analyses, referring them to the current species and examining the appropriate type materials. Clade A contains specimens characterized by a small primary thallus (Table 2) and branched podetia, with many lengthwise fissures along them (Fig. 3A). As mentioned above, the chemistry of this clade is variable: atranorin only; atranorin and fumarprotocetraric acid; atranorin, fumarprotocetraric and rangiformic acids; and atranorin and rangiformic acid, but psoromic acid or norstictic acid are never present. The morphological characters of these specimens are similar to those of the *Cladonia cariosa* lectotype and we consider that this clade represents *Cladonia cariosa* s.str. Additional characters for this taxa have been found, such as the presence of a thick epinecral layer above the cortex in the primary thallus and a smooth or fissured surface (Fig. 2B).

The specimens within clade B have large squamules, often prostrate and cracked. The podetia (present only in two of the studied samples) are corticate, with areolate zones, and slightly fissured (Fig. 3D). The chemical variability of this clade includes: atranorin only; atranorin together with norstictic acid; atranorin and fumarprotocetraric acid; atranorin and psoromic acid. The morphology of these specimens is similar to the neotype of *C. symphycarpa*, and the four chemotypes described for *C. symphycarpa* (Huovinen *et al.* 1989) are present in this clade. We consider clade B as being *C. symphycarpa* s.str. Additional taxonomically useful anatomic characters have been found. The squamule surface is smooth in young parts, while in older zones (middle and inferior zone of the squamules) some wide, shallow fissures can be observed using SEM (Fig. 2C). The specimens containing psoromic acid do not form
a monophyletic clade, which is consistent with a taxonomic concept that includes *C. dahliana* as a chemotype within *C. symphycarpa*.

Clade C included all specimens identified based on morphology as *C. acuminata*, supporting that this species is monophyletic. The specimens have large squamules and sorediate, mostly unbranched podetia, that can be rarely dichotomously branched near

FIG. 2. *Cladonia cariosa* group, anatomy of the primary thallus in the different clades. A, C, E & G, SEM micrographs of squamule surfaces; B, D, F & H, transverse sections of squamules; A & B, clade A (e = epinecral layer); C & D, clade B; E & F, clade C; G & H, clade D. Scales: A, C, E & G = 100 μm; B, D, F & H = 50 μm. In colour online.

TABLE 5. *Chemical variation found in the* Cladonia *specimens examined*

ATR	FUM	NST	PSO	RANG	ZEO	*C. cariosa*	*C. symphycarpa*	*C. acuminata*	*C. subcariosa*	*C. latiloba*
+						18	3	1	—	—
+					+	3	—	—	—	—
+	+					10	3	—	—	—
+	+			+		3	—	—	—	—
+		+			+	9	38	7	1	1
+		+				—	1	—	—	—
+			+			3	10	2	—	—
+			+		+	1	3	—	—	—
+				+		2	—	—	—	—
		+				—	7	—	—	—
			+			—	4	1	—	—
+	+	+				—	1	—	—	—
	+					—	—	—	1	—

ATR = atranorin, FUM = fumarprotocetraric acid, NST = norstictic acid, PSO = psoromic acid, RANG = rangiformic acid and ZEO = zeorin.

the tips (Fig. 3C), as already described for this species (Ahti 2000; Ahti & Hammer 2002). The three chemotypes found for this taxon are present in our sampling. The three *C. acuminata* chemotypes form a single monophyletic group. This clade includes specimens originating from North America with the three chemotypes, along with a European sample which contains atranorin and norstictic acid. Consequently, we interpret *C. acuminans* as a synonym of *C. acuminata*. Additionally, *C. acuminata* differs from the other species in the areolate-verrucose cortical surface (Fig. 2E). A specimen from Spain that only had a primary thallus is included in this clade. The squamules of this sample are morphologically and anatomically similar to those of the other samples in the clade. This specimen extends the range in Europe of *C. acuminata*, the southern limit of which in Europe was in Tyrol (Nimis 1993).

Clade D includes specimens with a primary thallus consisting of large squamules, similar in size to those of *C. symphycarpa*, but with podetia (Fig. 3B) similar to those of *C. cariosa* (with many fissures). Chemically this clade is also variable. It includes the following chemotypes: atranorin and norstictic acid; atranorin and psoromic acid; atranorin and fumarprotocetraric acid; and atranorin and zeorin. The specimens in this clade are an intermediate morphotype between *C. cariosa* and *C. symphycarpa*. This putative species has squamule surfaces that are strongly fissured (Fig. 2G) and lacking an epinecral layer. Furthermore, while the other species occur on calcareous substrata, these samples are found on acid substrata at an altitude above 1000 m. No taxonomic conclusion is made here concerning this clade because Vainio (1887) described several taxa in the group and the type materials of these names need to be examined before taxonomic conclusions can be drawn.

One specimen (*Cladonia symphycarpa* sp.lat. 4) could not be assigned to any of the four major clades (Fig. 1) and may represent another species in the group. Additional studies including more samples of this group are needed to evaluate the taxonomic status

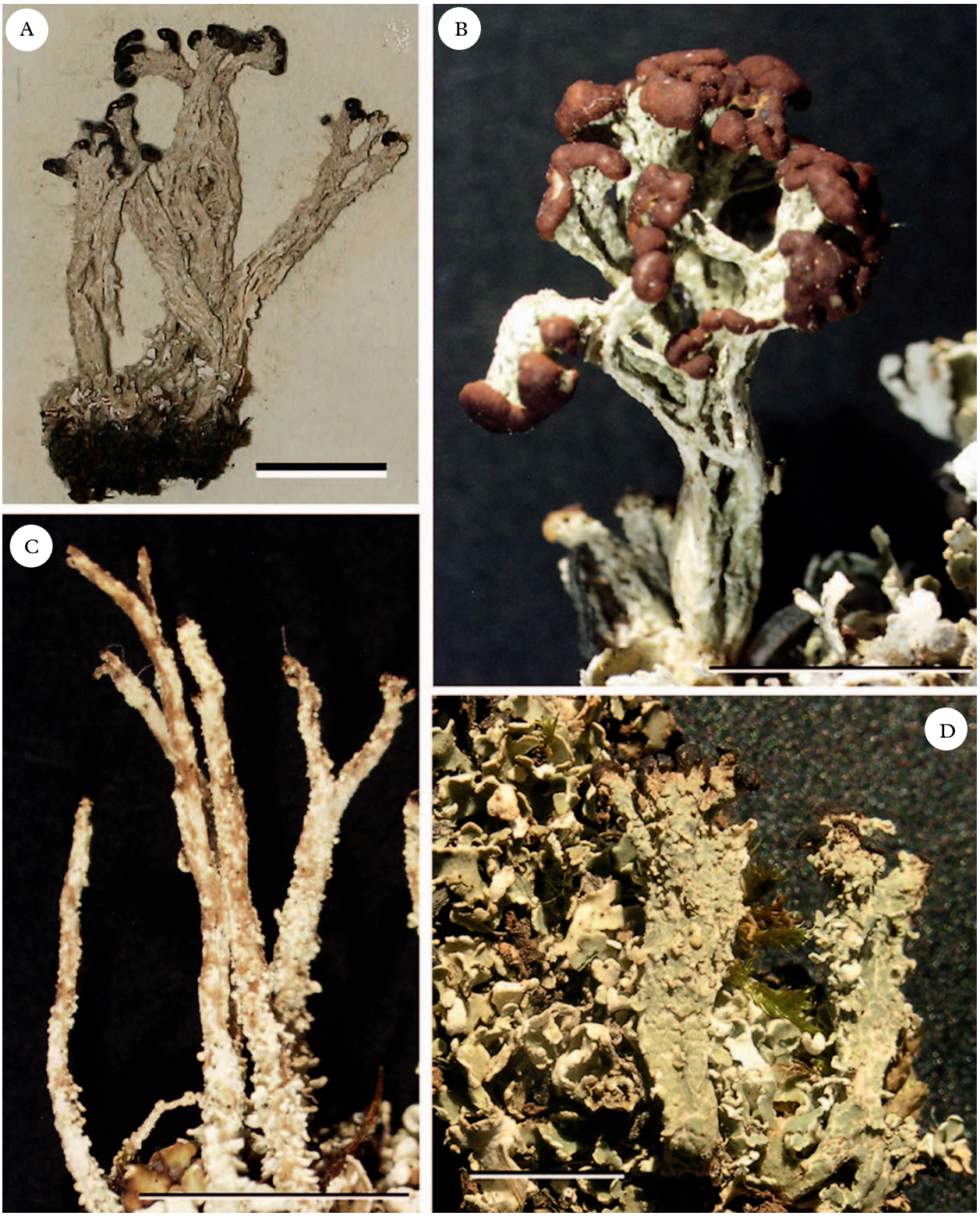

FIG. 3. Morphology of *Cladonia* species studied. A, *Cladonia cariosa* (lectotype); B, *C. cariosa* s. lat. (MACB 93018); C, *C. acuminata* (*T. Ahti* 63278); D, *C. symphycarpa* (MACB 101124). Scales = 5 mm. In colour online.

of this specimen. In these future studies we will try to include more specimens and the chemotype of *C. cariosa*, containing atranorin and homosekikaic acid that was described from North America and Greenland (Harris 1975; Bültmann & Lünterbusch 2008).

This study suggests that the *C. cariosa* group contains a greater number of species than was traditionally recognized, and that there are subtle morphological differences among them.

We are grateful to Professor Teuvo Ahti, who kindly sent us material of *C. acuminata*, and the curators of the herbaria B, BG, H, L, S and UPS for loans of specimens. Fátima Durán is thanked for technical help. The study was supported by the Spanish Ministry of Science and Technology (project CGL2007-66734-C03-01/BOS) and Universidad Complutense–Comunidad de Madrid (Research Group 910773). R. P-B was supported by a predoctoral grant from the Spanish Ministry of Education.

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Appendix. The phylogenetic relationships of *Cladonia subcariosa* and *C. latiloba* with the *C. cariosa* group.

A matrix of ITS rDNA with 102 sequences from species included in the supergroup *Cladonia* (Stenroos *et al.* 2002) was constructed to infer phylogenetic relationships of *C. subcariosa* and *C. latiloba* with the *C. cariosa* group. Maximum Parsimony and Bayesian analyses were performed. The Bayesian analysis was carried out using the GTG+I+G model (this model was selected in MrModeltest as the best-fitting evolutionary model using the AIC criterion). The posterior probabilities of each branch were calculated by counting the frequencies of trees that were visited during the course of the MCMC analysis. Model parameters were estimated in each analysis for 10 000 000 generations sampled in 4 simultaneous chains, and every 1000th was saved into a file. The initial 1000 trees were discarded as burn-in. Using the "sumt" command of MrBayes, the 50% majority-rule consensus tree was calculated from 18 000 trees sampled after reaching likelihood convergence to calculate the posterior probabilities of the tree nodes.

The matrix contained 587 characters, 186 of them parsimony informative. MP analyses generated 1000 equally parsimonious trees, 795 step long, CI = 0·4579, RI = 0·7810 and RC = 0·3576. The Bayesian analysis yielded a tree with a likelihood value of Ln = −5435·17. *Cladonia subcariosa* and *C. latiloba* are not closely related to the *C. cariosa* group (Fig. A1). *Cladonia subcariosa* appears to be closely related to *C. caespiticia* with high support, while the relationship of *C. latiloba* is not resolved.

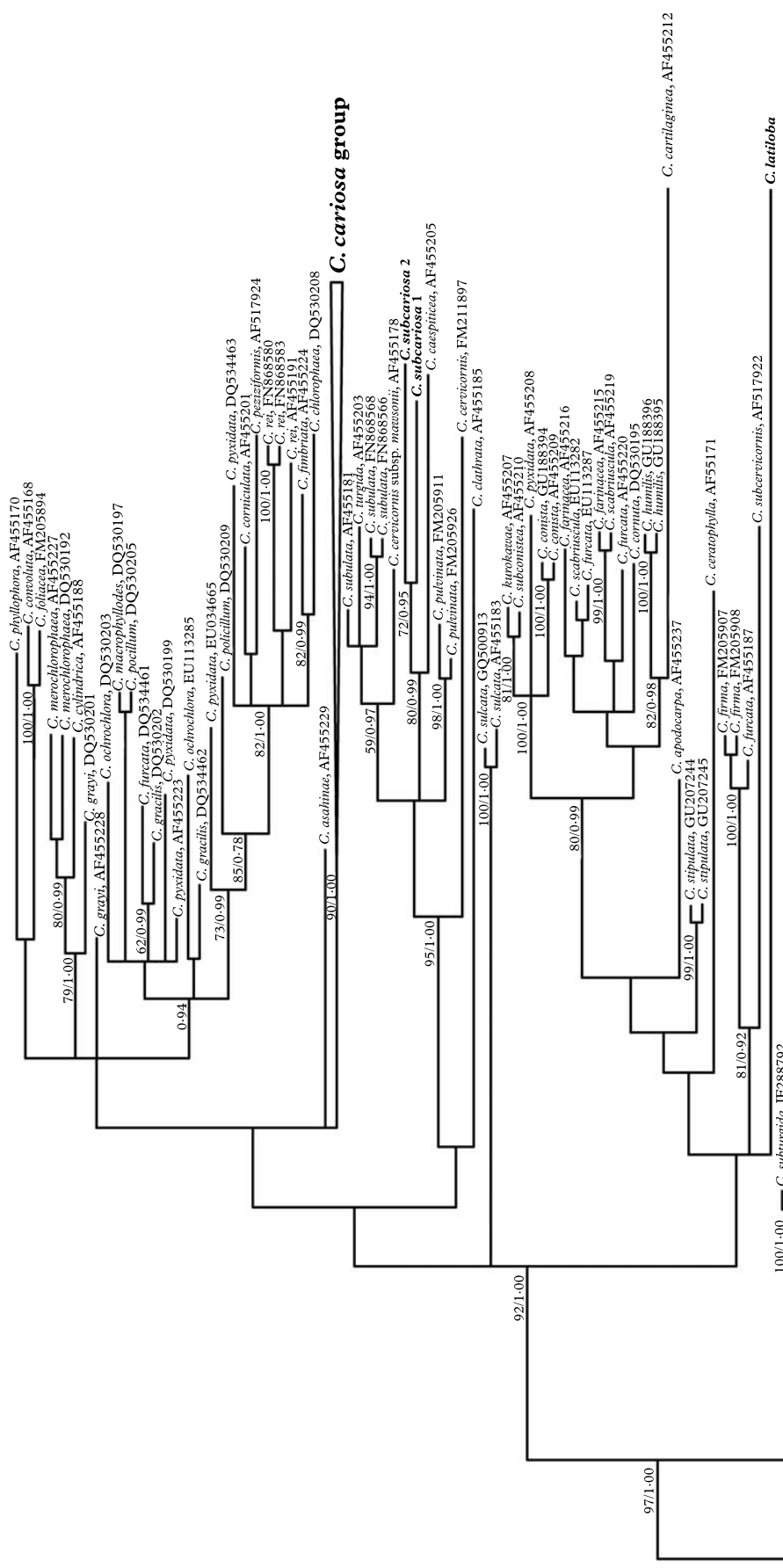

FIG. A1. Phylogenetic placement of *Cladonia subcariosa* and *C. latiloba* in the supergroup *Cladonia*. The 50% majority-rule consensus tree from a Bayesian analysis based on ITS rDNA. The boostrap values of MP analysis and posterior probability of Bayesian analysis are indicated on the branches.

Phenotypical plasticity and homoplasy complicate species delimitation in the *Cladonia gracilis* group (Cladoniaceae, Ascomycota)

V

ARTÍCULO V

Phenotypical plasticity and homoplasy complicate species delimitation in the *Cladonia gracilis* group (Cladoniaceae, Ascomycota)

Raquel Pino-Bodas, Ana Rosa Burgaz, María P. Martín & H. Thorsten Lumbsch

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La delimitación de especies en el grupo de *Cladonia gracilis* ha sido difícil durante mucho tiempo debido a la gran variabilidad morfológica de los taxones. Los caracteres utilizados para distinguir los taxones de este grupo son: 1) la presencia de podecios sorediados o corticados, 2) el grosor de la pared de los podecios 3) la presencia de escifos y su diámetro 4) la presencia de escuámulas sobre los podecios y 5) el color de la base de los podecios. La distribución geográfica y los requerimientos ecológicos a veces son de gran ayuda para distinguir los taxones, pero algunos de ellos coexisten en varias regiones, lo que dificulta las identificaciones. El presente estudio aborda la delimitación de especies dentro de este grupo examinando numerosos especímenes de los taxones que se aceptan en la actualidad: *Cladonia coniocraea, C. cornuta subsp. cornuta, C. cornuta* subsp. *groenlandica, C. ecmocyna* subsp. *ecmocyna, C. ecmocyna* subsp. *intermedia, C. gracilis* subsp. *gracilis, C. gracilis* subsp. *elongata, C. gracilis* subsp. *tenerrima, C. gracilis* subsp. *turbinata, C. gracilis* subsp. *vulnerata, C. macroceras, C. maxima*, y *C. ochrochlora,* empleando el reconocimiento de especies filogenéticas mediante concordancia genealógica. Se realizaron reconstrucciones filogenéticas de máxima parsimonia, máxima verosimilitud y bayesiana usando secuencias de DNA de ITS rDNA, IGS rDNA, *rpb2* y *ef1α*. Los resultados indican que el grupo de *C. gracilis* es monofilético, pero que los taxones actualmente aceptados no forman grupos monofiléticos a excepción de *C. ecmocyna* y *C. cornuta* subsp. *cornuta*. Los diferentes análisis realizados sugieren que la clasificación incompleta de linajes y los eventos esporádicos de recombinación son responsables de la ausencia de apoyo en la filogenia del grupo. Nuestros datos sugieren de forma clara que

C. coniocraea, C. cornuta subsp. *groenlandica* y *C. ochrochlora* son coespecíficas, siendo *C. coniocraea* el nombre válido. Los caracteres morfológicos en este grupo resultaron ser altamente homoplásicos, causando, junto con la plasticidad fenotípica de los taxones, las dificultades en la delimitación de especies en el grupo de *C. gracilis*.

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ORIGINAL ARTICLE

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Phenotypical plasticity and homoplasy complicate species delimitation in the *Cladonia gracilis* group (Cladoniaceae, Ascomycota)

Raquel Pino-Bodas · Ana Rosa Burgaz · María P. Martín · H. Thorsten Lumbsch

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Abstract Species delimitation in the *Cladonia gracilis* group has long been known to be difficult due to morphological variability of taxa. The present study addresses the circumscription of species within this group, examining a number of specimens of the currently accepted taxa *Cladonia coniocraea*, *C. cornuta* subsp. *cornuta*, *C. cornuta* subsp. *groenlandica*, *C. ecmocyna* subsp. *ecmocyna, C. ecmocyna* subsp. *intermedia, C. gracilis* subsp. *gracilis, C. gracilis* subsp. *elongata, C. gracilis* subsp. *tenerrima, C. gracilis* subsp. *turbinata, C. gracilis* subsp. *vulnerata, C. macroceras, C. maxima,* and *C. ochrochlora* using genealogical concordance phylogenetic species recognition. We employed maximum parsimony, maximum likelihood and Bayesian methods of phylogenetic reconstructions based on DNA sequences of ITS rDNA, IGS rDNA, *RPB2* and partially *EF1-α* regions. Our results indicate that the *C. gracilis* group is monophyletic but that most currently accepted taxa do not form monophyletic groups, with the exception of *C. ecmocyna* and *C. cornuta* subsp. *cornuta.* Different tests suggest that incomplete lineage sorting and sporadic recombination events are responsible for a phylogeny that largely lacks support. Our data also strongly suggest that *C. coniocraea, C. cornuta* subsp. *groenlandica,* and *C. ochrochlora* are conspecific, with *C. coniocraea* being the oldest available name. The morphological characters in the group are shown to be highly homoplasious, causing, in tandem with phenotypical plasticity of the taxa, the difficulties in delimiting species in the *C. gracilis* group.

Keywords Genealogical concordance phylogenetic species recognition · Lichens · Morphology · Taxonomy · Variability

R. Pino-Bodas (✉) · A. R. Burgaz
Departamento de Biología Vegetal 1, Facultad de Biología,
Universidad Complutense de Madrid,
28040 Madrid, Spain
e-mail: rpino@bio.ucm.es

M. P. Martín
Departamento de Micología, Real Jardín Botánico,
CSIC,
28014 Madrid, Spain

H. T. Lumbsch
Department of Botany, The Field Museum,
1400 S. Lake Shore Drive,
Chicago, IL 60605, USA

Introduction

Understanding morphological variation in the different groups of organisms is a key to identify diagnostic characters allowing for taxon delimitation. Nevertheless, this task is hindered by phenotypical plasticity, i.e., the ability of a genotype to express different phenotypes as a response to environmental conditions. This fact is widely known for many groups of organisms (Sultan 1987; Lortie and Aarssen 1996; Trussell 1996; Schichting 2002; Whitman and Ananthakrishnan 2008; Hollander and Butlin 2010), including lichenized fungi (Nash et al. 1990; Pintado et al. 1997; Rikkinen 1997). In order to assess the phenotypical plasticity effect, it is required to study the phenotypical response of a given genotype in an environmental gradient. These kinds of experiments have been carried out in plants (Briggs 1964; Davis 1983; Pigliucci et al. 1999; Kaplan 2002a; Canfield et al. 2008), which are usually easy to grow. Growing lichenized fungi, however, is not an easy task, which has seriously limited this kind of studies in lichens. Currently, analyses based on DNA sequences permit to assess whether morphological variation is related to genetic differences or not, the

same way they have permitted to evaluate the significance of the reproductive type in numerous so-called species pairs (Lohtander et al. 1998a, b; Myllys et al. 1999, 2001; Articus et al. 2002; Buschbom and Mueller 2006).

Species within the genus *Cladonia* are known to be morphologically variable, which explains taxonomic problems remaining in some species groups of *Cladonia* (Ahti and Hammer 2002), in spite of being one of the most studied macrolichen genera. The high morphological variability of *Cladonia* species is probably an effect of the phenotypical plasticity induced by factors such as exposure to light, temperature or humidity (Ahti 2000). The *Cladonia gracilis* group, treated as "subgroup *Graciles*" by Stenroos et al. (2002) is a monophyletic group (Fontaine et al. 2010) formed by *C. gracilis* (L.) Willd., *C. ecmocyna* Leight., *C. cornuta* (L.) Hoffm., *C. squamosissima* (Müll. Arg.) Ahti, *C. coniocraea* (Flörke) Spreng., *C. ochrochlora* Flörke, *C. maxima* (Asahina) Ahti and *C. macroceras* (Delise) Hav. The characters used to distinguish the species in the group include: 1) presence of partially sorediate or entirely corticate podetia, 2) width of the podetial wall, 3) presence of squamules on the podetium, 4) presence of scyphi and their diameter, and 5) color of the podetial base. In this group, the secondary metabolites are relatively uniform, with most species containing only fumarprotocetraric acid. Some taxa regularly contain atranorin, while in some others atranorin appears only in certain specimens. In the *C. gracilis* group, species delimitations are especially difficult since taxa are morphologically similar, because most of the morphological characters show a considerable variation (Ahti 1980a). These difficulties have led to the necessity of using a combination of character states (Ahti 1980a) in order to distinguish the taxa. Distribution differences and ecological requirements are sometimes a great help to differentiate between taxa, but wherever several taxa coexist some can be difficult to distinguish. An example to illustrate this is the distinction of *Cladonia coniocraea* and *C. ochrochlora*, two morphologically similar species, that have ecological and distributional differences. *Cladonia ochrochlora* grows in N and S hemispheres with oceanic tendency while *C. coniocraea* appears only in North America and Eurasia (Ahti 2000; Ahti and Hammer 2002). As such, the species rank of these taxa has been questioned (Poelt and Vězda 1981; Nimis 1993; Wirth 1995).

Fontaine et al. (2010) demonstrated, using ITS rDNA and PKS sequence data, that the group is monophyletic while the currently accepted morphospecies are not, with the exception of *C. maxima*. Further, the clades that were found in this analysis only got weak support values. Hence, to further understand the species delimitation in the *C. gracilis* group we gathered DNA sequence data using three loci (ITS rDNA, IGS rDNA, *RPB2*). Additionally, we focused on the delimitation of *C. coniocraea* and *C. ochrochlora,* for which we used four genetic markers (*EF1*-α added) to assess the taxonomical status of these taxa. We applied genealogical concordance phylogenetic species recognition (Taylor et al. 2000) in this study.

Material and methods

Taxon sampling

From a total of 770 studied collections from different herbaria (B, BRA, FH, H, L, MACB, S, UPS), 115 were selected for the molecular study. It was intended that all the taxa in the group of *C. gracilis* were represented. We sampled specimens of *C. coniocraea, C. cornuta* subsp. *cornuta*, *C. cornuta* subsp. *groenlandica*, *C. ecmocyna* subsp. *ecmocyna*, *C. ecmocyna* subsp. *intermedia*, *C. gracilis* subsp. *gracilis*, *C. gracilis* subsp. *elongata*, *C. gracilis* subsp. *tenerrima*, *C. gracilis* subsp. *turbinata*, *C. gracilis* subsp. *vulnerata*, *C. macroceras*, *C. maxima*, and *C. ochrochlora*. However, there are taxa, which apparently belong to this complex, such as *Cladonia alinii* Trass, *C. cinerella* Ahti, *C. ecmocyna* subsp. *occidentalis* Ahti, *C. fenestralis* Nuno, *C. gracilis* subsp. *valdiviensis* Ahti, and several Australasian species similar to *C. ochrochlora*, which were not analyzed. As outgroup we included the closely related species *C. rangiformis* and *C. thomsonii* following Stenroos et al. (2002) and based on our own unpublished data.

DNA extraction and PCR

Total DNA was extracted using DNeasy Plant Mini Kit (QIAGEN, Germany) following the manufacturer's instructions. The DNA was dissolved in 200 μl of buffer included in the kit. Three loci were amplified for all the samples: ITS rDNA, IGS rDNA and *RPB2*. A fourth region, *EF1*-α, was amplified in a subset including *C. coniocraea* and *C. ochrochlora* samples. The amplifications were carried out using the primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990), or alternatively SSU-1780-5'F/LSU-0012 (Piercey-Normore and DePriest 2001) for the ITS region; primers IGSf/IGSr (Wirtz et al. 2008) for the IGS rDNA region; RPB2-5F/RPB2-7cR (Liu et al. 1999) and RPB2dRaq/RPB2rRaq (Pino-Bodas et al. 2010) for the *RPB2* region; and primers CLEF-3F/CLEF-3R (Yahr et al. 2006) for the *EF1*-α region. PCRs were carried out with Ready-to-Go-PCR Beads (GE Healthcare Life Sciences, UK). Amplifications were prepared for a 25 μl final volume. The thermal cycling parameters used for amplifications of ITS rDNA, *RPB2* and *EF1*-α were those of

Pino-Bodas et al. (2010) and the program used for IGS rDNA region was the same used to amplify *RPB2* in Pino-Bodas et al. (2010). The PCR products were purified using the QIAquick Gel extraction Kit (QIAGEN) or ExoSAP-IT (USB Corporation, OH, USA).

The sequencing reactions were done at the Secugen S. L. (Centro de Investigaciones Biológicas [CIB], Madrid, Spain) or Macrogen (Korea) sequencing service (www.macrogen.com) with the same primers used for the PCR. The SequencherTM program (Gene Codes Corporation, Inc, Ann Arbor, Michigan, USA) was used to assemble the consensus sequences from the two strands of each sequencing reaction.

Phylogenetic analyses

The alignments were produced manually with SE-AL v2.0a11 (Rambaut 2002) for each locus separately. Phylogenetic analyses were done using different matrices: 1) on each matrix corresponding separately to each locus, 2) on a concatenated matrix including three loci that contained all samples, and 3) a concatenated matrix including four loci that contained a subset of the samples (*C. coniocraea* and *C. ochrochlora* only). All matrices were analyzed by maximum parsimony (MP) analyses. MP analyses were performed using PAUP* version 4.0.b.10 (Swofford 2003), using the heuristic search with 1000 random taxon-addition replicates with TBR branch swapping and MulTrees option in effect, equally weighted characters and gaps treated as missing data. For the confidence analysis, the bootstrap (Felsentein 1985) was applied, with 1,000 replicates, using the heuristic option with the same settings as the MP search. Congruence among loci was tested following Lutzoni et al. (2004): each clade with more than 75% bootstrap support was scanned for conflict among loci and individual sequences causing conflict were excluded from the data matrix (Appendix 2). We considered the existence of a conflict whenever a clade was supported with a bootstrap (more than 75%) in a locus, but it was not supported in other locus and the individual sequences of this clade are part of another clade with bootstrap support ≥ 75 %.

MrModeltest (Nylander 2004) was used to select the most appropriate nucleotide substitution model for each locus, using the AIC criterion. In addition to MP, the combined dataset and the subsets were also analyzed, using maximum likelihood (ML) and Bayesian analysis. ML analyses were implemented with RaxML 7.04 (Stamatakis 2006), the combined dataset was divided in five partitions (ITS rnDNA, IGS rnDNA and each of three codon positions of *RPB2*), assuming the GTRGAMMA model for all partitions. The dataset of the subset with four loci was divided into eight partitions, five as described above and each of three codon positions of *EF1-α*. The Bayesian analysis was carried out using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). The combined dataset was divided in five partitions: ITS rnDNA (optimal model, K80 + I), IGS rnDNA (HKY + G) and each of three codon positions of *RPB2* (each K80 + I). The dataset of the subset with four loci was divided into eight partitions, five as described above and each of three codon positions of *EF1-α* (each SYM + I). The posterior probabilities were approximated by sampling trees using Markov Chain Monte Carlo (MCMC). Two simultaneous runs with 20,000,000 generations, each starting with a random tree and employing 4 simultaneous chains were executed. Every 1,000th tree was saved into a file. Tracer v. 1.0 (http://evolve.zoo.ox.ac.uk/software.html?id=tracer) and AWTY (Nylander et al. 2008) were used to determine when the chains reached the stationary stage and the number of generations that should be discarded as burn-in. The 50% majority-rule consensus tree was calculated using the "sumt" command of MrBayes, deleting the first 1,000,000 generations (i. e. the first 1,000 trees). The matrices and trees have been deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S11906).

The ILD test (Farris et al. 1994) as implemented in PAUP* was used to estimate the discordance among loci. The analyses were performed with 1,000 replications and a value of $p<0.001$ was considered significant, following Cunningham (1997). Then, the ILD test was conducted in several reduced matrices in order to evaluate the influence of the different data subsets. The outgroup taxa were excluded in all cases.

Hypothesis testing

The following phylogenetic hypotheses of monophyly were tested: 1) *C. ochrochlora,* 2) *C. coniocraea,* 3) *C. cornuta* subsp. *cornuta* plus *C. cornuta* subsp. *groenlandica,* 4) the sorediate taxa (*C. coniocraea*, *C. ochrochlora* and *C. cornuta*), 5) *C. gracilis* subsp. *gracilis,* 6) *C. gracilis* subsp. *turbinata*. 7) *C. gracilis* subsp. *elongata* is monophyletic. Alternative hypothesis tests included the Shimodaira-Hasegawa test (SH) (Shimodaira and Hasegawa 1999) and expected likelihood weight (ELW) (Strimmer and Rambaut 2002) that were performed using TREE-PUZZLE 5.2 (Schmidt et al. 2002).

Detecting possible recombination

Two methods were used to assess the interlocus recombination rate: the Index of Association (I_A) (Brown et al. 1980; Maynard Smith et al. 1993) and the Parsimony Tree Length Permutation Test (PTLPT) (Burt et al. 1996). The I_A was calculated in MULTILOCUS 1.3b (Agapow and Burt 2001), using standardization for the covariances (rd). This modification removes the dependency of the number of loci

analyzed (Zhang et al. 2010). The positions in the same region were considered as a single locus. A significance test was performed with 1,000 randomizations of the data to determine whether the association index was significantly different from zero. For the calculation of PTLPT, 1,000 artificially recombined replicas were generated using MULTILOCUS and PAUP* was used to calculate the tree lengths for these 1,000 replicas.

Additionally, several tests for assessing intralocus recombination were carried out. In RDP3 program (Martin et al. 2005), the followings tests were performed: Bootscanning (Salminen et al. 1995); Chimaera (Posada and Crandall 2001); GENECONV (Padidam et al. 1999); MaxChi (Maynard Smith 1992); RDP (Martin et al. 2005); SiScan (Gibbs et al. 2000); and 3SEQ (Boni et al. 2007). In SPLITSTREE4 (Huson and Bryant 2006), the PHI test (Bruen et al. 2006) was implemented.

Homoplasy of phenotypical characters

An evaluation of the taxonomic utility of phenotypical characters was done following Lumbsch (2004). The homoplasy for each character was assessed by the consistency index (CI) and retention index (RI), using Mesquite 2.74 (Maddison and Maddison 2007), calculated over the strict consensus tree of the maximum parsimony search based on the combined dataset. The matrix with the morphological and chemical characters and their respective states for every sample is shown in Appendix 1.

Morphological study of *C. coniocraea* and *C. ochrochlora*

The morphological and anatomical data of *C. coniocraea* and *C. ochrochlora* were analyzed by principal component analysis (PCA) using a correlation matrix in SPSS 17.0.

Table 1 Samples included in phylogenetic analyses with GenBank accession number

Species	Locality and collection	ITS	*RPB2*	IGS	*EF1-α*
C. coniocraea 1	USA, New Hampshire, *M. Schmull* (FH 259371)	JN811378	JN811336	JN811344	JN811437
C. coniocraea 2	Finland, Etelä-Häme, Päijänne, *V. Haikonen* (H)	JN811379	JN811337	JN811345	—
C. coniocraea 3	Russia, Karelian Republic, *P. Uotila* (H)	JN811380	JN811338	JN811346	JN811438
C. coniocraea 4	Russia, Sakha Republic, *T. Ahti & P. A. Timofeev* (H)	JN811381	JN811339	JN811347	JN811439
C. cornuta subsp. *cornuta* 1	Finland, Etelä-Häme, Päijänne, *V. Haikonen* (H)	JN811383	JN811426	JN811348	—
C. cornuta subsp. *cornuta* 2	Finland, Uusimaa, *R. Pino-Bodas* (MACB 101646)	JN811385	JN811428	JN811350	—
C. cornuta subsp. *groenlandica* 1	USA, Alaska, *K. Dillman* (H)	JN811384	JN811427	JN811349	JN811436
C. ecmocyna 1	Norway, Østlandet, *S. Rui & E. Timdal* (H)	JN811399	JN811423	JN811365	—
C. ecmocyna 2	Spain, Burgos, *A. R. Burgaz* (MACB 101650)	JN811397	JN811424	JN811352	—
C. ecmocyna 3	Spain, Madrid, *A. R. Burgaz* (MACB 101649)	JN811398	JN811425	JN811353	—
C. gracilis subsp. *gracilis* 1	Spain, Palencia, *A. R. Burgaz* (MACB 94216)	JN811386	JN811412	JN811354	—
C. gracilis subsp. *gracilis* 2	Spain, Guadalajara, *A. R. Burgaz* (MACB 95195)	JN811387	JN811413	JN811355	—
C. gracilis subsp. *gracilis* 3	Finland, Uusimaa, *V. Haikonen* (H)	JN811390	JN811419	JN811361	—
C. gracilis subsp. *gracilis* 4	Finland, Kymenlaakso, *V. Haikonen* (H)	JN811392	JN811420	JN811362	—
C. gracilis subsp. *gracilis* 5	Finland, Uusimaa, *R. Pino-Bodas* (MACB 101647)	JN811394	JN811422	JN811364	—
C. gracilis subsp. *elongata* 1	Russia, Murmansk Region, *M. A. Fadeeva* (H)	JN811391	JN811418	JN811360	—
C. gracilis subsp. *elongata* 2	Sweden, Uppland, *G. Thor* (UPS L167919)	JN811388	JN811414	JN811356	—
C. gracilis subsp. *turbinata* 1	Russia, Leningrad Region, *T. Ahti* (H)	JN811389	JN811415	JN811357	—
C. gracilis subsp. *turbinata* 2	Finland, Pirkanmaa, *V. Haikonen* (H)	JN811393	JN811421	JN811363	—
C. gracilis subsp. *vulnerata* 1	USA, Alaska, *K. Dillman* (H)	JN811395	JN811417	JN811358	—
C. gracilis subsp. *vulnerata* 2	USA, Alaska, *S. S. Talbot & W. B. Schofield* (H)	JN811396	JN811416	JN811359	—
C. macroceras 1	Andorra, Soldeu, *A. R. Burgaz* (MACB 94200)	JN811382	JN811411	JN811351	—
C. ochrochlora 1	Spain, Lugo, *A. R. Burgaz* (MACB 95427)	JN811374	JN811407	JN811340	JN811440
C. ochrochlora 2	Portugal, Beira Alta, *A. R. Burgaz* (MACB 94635)	JN811375	JN811408	JN811341	JN811441
C. ochrochlora 3	Finland, Uusimaa, *R. Pino-Bodas* (MACB 101648)	JN811376	JN811409	JN811342	JN811442
C. ochrochlora 4	Unknown, *L. R. Sharma*	JN811377	JN811410	JN811343	JN811443
C. rangiformis 1	Spain, Menorca, *A. R. Burgaz* (MACB 96193)	JF288803	JF288838	JN811366	JN811444
C. rangiformis 2	Netherlands, Zuid-Holland, *H. Van der Goes et al.* (H)	JN811400	JN811429	JN811367	—
C. rangiformis 3	Sweden, Öland, *R. Skytén* (H)	JN811401	JN811430	JN811368	—
C. thomsonii 1	Russia, Krasnoyarsk, *M. Zhurbenko* (H)	JN811402	JN811431	JN811369	—

(SPSS Inc., Chicago, IL, USA). In the analysis, the key characters for the discrimination of *C. coniocraea* and *C. ochrochlora* (Hammer 1991; Ahti 2000; Ahti and Hammer 2002) were included, whenever they, or their logarithmic transformation, were normally distributed as indicated by a Kolmogorov-Smirnov test: squamule length, squamule thickness, squamule incisions, podetial length, podetial thickness, podetial basal cortex thickness and length. Soredium size, squamule width and podetial diameter did not fulfill the normality criterion and were excluded from the analysis.

Results

Phylogenetic reconstructions

The DNA of a total of 115 specimens was isolated, but not all the specimens were successfully amplified for one or more loci. Old material and gamma irradiated material for preservation proved difficult to amplify. Sixty two sequences for ITS rDNA, 77 for IGS and 43 for *RPB2* were obtained. Only in 30 samples were the amplifications of all three regions achieved; 4 of them were incongruent (appendix 2) according to the method of Lutzoni et al. (2004) and were excluded from the combined analyses (Table 1). In the MP analyses of each separate region, the relationships in the ingroup were mostly unresolved. In the consensus tree for the ITS rDNA region (not shown), three clades appeared. One of them contained *C. ecmocyna* samples; another with *C. cornuta* subsp. *cornuta* samples; and a third one containing the samples of *C. gracilis* subsp. *vulnerata*. But only the *C. cornuta* subsp. *cornuta* clade was strongly supported (=95% bootstrap support [BP]). In the resulting tree for IGS rDNA analysis, the same clades obtained for ITS rDNA analysis were found, and the clades of *C. ecmocyna* and *C. gracilis* subsp. *vulnerata* received 76 and 87% BP support respectively, while *C. cornuta* subsp. *cornuta* received only low support (63% BP). In the *RPB2* analysis, only one, strongly supported (100% BP) clade containing *C. coniocraea, C. ochro-*

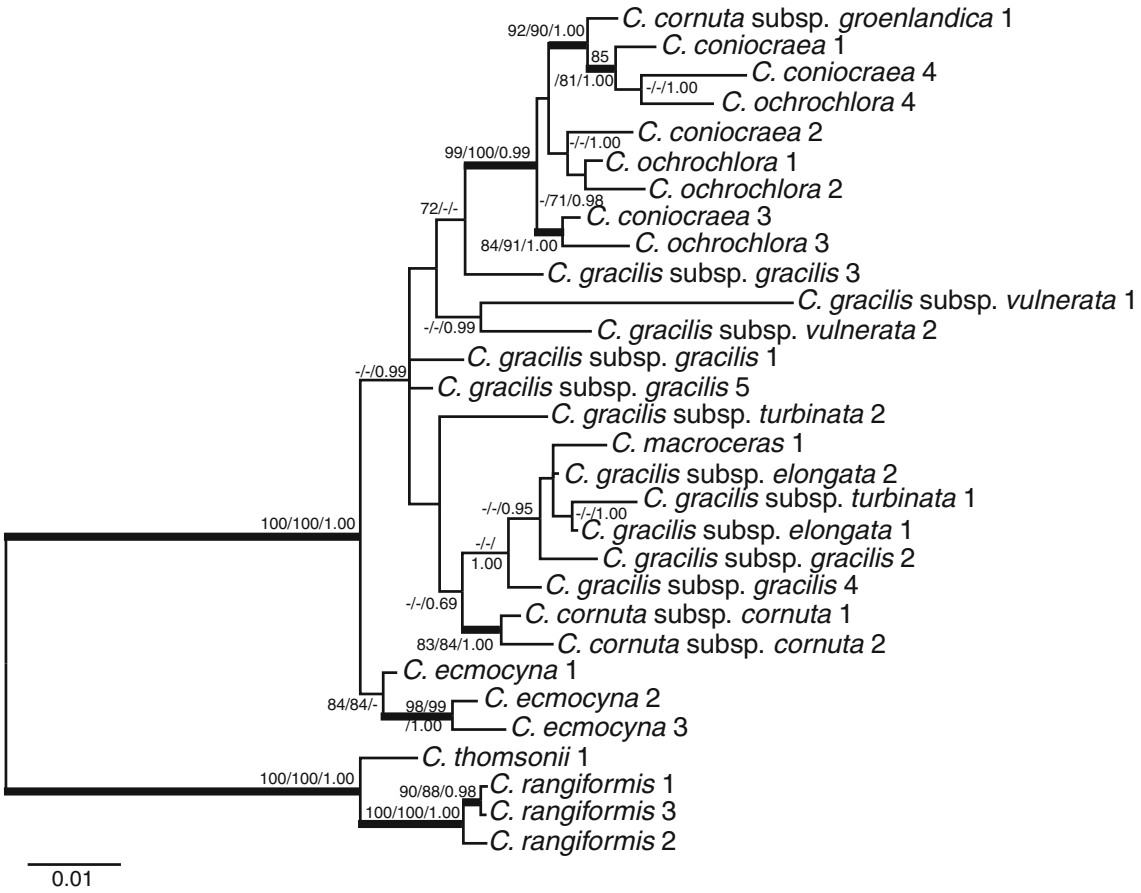

Fig. 1 Phylogeny of the *C. gracilis* group. 50% Majority Rule Bayesian tree based on a combined data set including ITS rDNA, IGS and *RPB2*. Branches supported with posterior probability ≥0.95 and bootstrap >70% are indicated in bold. Bootstrap value >70% for MP/ Bootstrap value >70% for ML/posterior probability >0.95 for Bayesian analysis at branches

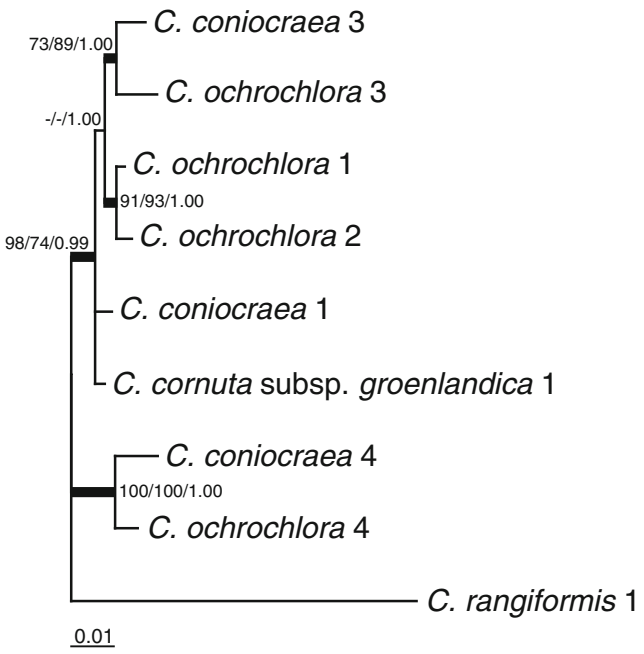

Fig. 2 Phylogeny of the *Cladonia coniocraea* and *C. ochrochlora* complex. 50% Majority rule Bayesian tree based on ITS rDNA, IGS, *RPB2* and *EF1-α*. Branches supported with posterior probability ≥0.95 and bootstrap >70% are indicated in bold. Bootstrap value >70% for MP/ Bootstrap value >70% for ML/posterior probability >0.95 for Bayesian analysis at branches

chlora and *C. cornuta* subsp. *groenlandica* specimens was found.

The combined dataset of 30 sequences (26 ingroup and 4 outgroup OTUs) included 1823 unambiguously aligned sites (ITS: 578, IGS: 380, and *RPB2*: 865) and included 174 parsimony informative positions. The MP analysis yielded 9 equally parsimonious trees of 428 steps, CI=0.738, RI=0.829 and RC=0.617. ML analysis yielded a most likely tree with a likelihood value of LnL= −5118.76, while the mean likelihood of the Bayesian tree sampling was LnL=−5201.09. MP, ML and Bayesian analyses rendered a similar topology, so only the Bayesian tree is shown here (Fig. 1). The *C. gracilis* group, including *C. ecmocyna*, *C. cornuta*, *C. ochrochlora*, *C. coniocraea, C. macroceras* and *C. gracilis* sensu lato, is well-supported in all three analyses. Two clades have strong support in all three analyses: 1) a clade containing samples of *C. coniocraea*, *C. ochrochlora* and one sample of *C. cornuta* subsp. *groenlandica,* all intermingled; and 2) a clade consisting of two *C. cornuta* subsp. *cornuta* specimens. The *C. gracilis* subsp. *vulnerata* specimens form a monophyletic group that only receives support in the Bayesian analysis. *Cladonia ecmocyna* specimens form a monophyletic clade basal to the remaining taxa of the *C. gracilis* group. This clade is well supported in MP and ML analyses, but lacks support in the Bayesian analysis (Fig. 1). Specimens of *C. gracilis* subsp. *turbinata*, *C. gracilis* subsp. *gracilis* and *C. gracilis* subsp. *elongata* do not form monophyletic groups.

The subset focusing on *C. coniocraea* and *C. ochrochlora* contained 2433 unambiguously aligned nucleotide sites (ITS: 578, IGS: 380, *RPB2*: 865 and *EF1-α*: 610), 2200 of which were constant, and 59 were parsimony-informative. MP analysis yielded one tree, 268 steps long, with a value CI= 0.899 and RI=0.716. ML analysis generated a tree with LnL= −4717.16, while the mean likelihood of the Bayesian analysis was LnL=−4882.01. The topology of the phylogenetic trees of the three analyses were similar, thus only the Bayesian tree is shown here (Fig. 2). The samples of *C. coniocraea* and *C. ochrochlora* were intermingled and did not form separate monophyletic groups.

The SH and ELW rejected six alternative hypotheses tested (Table 2). The monophyly of *C. gracilis* subsp. *elongata* was rejected by ELW but it was not rejected by SH. The results of ILD test are shown in Table 3. The test on complete matrix indicates incompatibilities among the three loci. When the analysis was run only with the *C. ochrochlora*/*C. coniocraea* subset, no evidence of heterogeneity among loci was found. In three data subsets, ITS and IGS were compatible (Table 3), but in the same subsets ITS-*RPB2* and IGS-*RPB2* were incompatible. In the remaining subsets, all genes were incompatible.

Table 2 Results of the alternative hypothesis tests. P-value of the constrasts given. Asterisks denote significant results

Hypothesis	SH	ELW
C. cornuta subsp. *cornuta* and *C. cornuta* subsp. *groenlandica* form a monophyletic clade	0.0100*	0.0000*
C. ochrochlora is monophyletic	0.0200*	0.0028*
C. coniocraea is monophyletic	0.0170*	0.0005*
C. cornuta subsp. *cornuta* forms a monophyletic clade with *C. ochrochlora* and *C. coniocraea*	0.0001*	0.0001*
C. gracilis subsp. *gracilis* is monophyletic	0.0010*	0.0000*
C. gracilis subsp. *turbinata* is monophyletic	0.0010*	0.0000*
C. gracilis subsp. *elongata* is monophyletic	0.0700	0.0391*

Table 3 Results of the ILD tests from comparisons of different loci in a series of matrices. Asterisks denote significant results

	ITS-IGS	ITS*RPB2*	IGS-*RPB2*
All samples	0.001*	0.001*	0.001*
Only *C. ochrochlora*/*C. coniocraea* clade included	0.810	0.029	0.204
C. ochrochlora/*C. coniocraea* clade excluded	0.010	0.001*	0.001*
C. ochrochlora/*C. coniocraea* and *C. ecmocyna* excluded	0.009	0.001*	0.001*
C. ochrochlora/*C. coniocraea*, *C. ecmocyna, C. cornuta* excluded	0.011	0.001*	0.001*
Only *C. gracilis* s.l. included	0.003	0.001*	0.001*
C. gracilis s.l. included but *C. gracilis* subsp. *vulnerata* excluded	0.003	0.001*	0.001*

Recombination

The results of the I_A analysis yielded a p-value=0.022, rejecting the null hypothesis of recombination. The PTLPT test revealed that the observed tree length was shorter than in the permutated data, also indicating the absence of recombination (Fig. 3).

No chimeric sequences were identified in any of the regions by the tests implemented in RDP3. However, the PHI test detected evidences of recombination in ITS (P=0.041) and *RPB2* (P=0.047), but not in IGS (P=0.539). The PHI test was repeated in ITS rDNA and *RPB2* matrices with all the missing data and gaps removed from them, but the results did not change.

Homoplasy of phenotypical characters

The results of CI and RI value for each character are summarized in Table 4. Most of the characters were found to be strongly homoplasious, with low values of CI and RI. The character corticate podetium has the lowest amount of homoplasy with a CI=0.5 and RI=0.9.

Morphological study of *C. coniocraea* and *C. ochrochlora*

The PCA analysis included 59 specimens of *C. coniocraea* and *C. ochrochlora*. The first three components explain 37.5%, 20.9% and 14.9% of the variance, respectively (73.5 cumulative variance). The analysis shows a continuous variation and does not distinguish the samples of *C. coniocraea* and *C. ochrochlora* (Fig. 4).

Discussion

Our analyses confirm previous studies (Fontaine et al. 2010) supporting the monophyly of the *C. gracilis* group. We included *C. gracilis* subsp. *vulnerata* and *C. cornuta* subsp. *groenlandica* in a molecular study for the first time and confirm that these taxa belong to the *C. gracilis* group. The studied loci generally offered poor resolution when analyzed separately with numerous polytomies or poorly supported clades. Only the analyses of the combined matrix partially resolved the phylogeny of the group. Only a few of the accepted morphospecies turned out to be monophyletic in our analyses. Fontaine et al. (2010) also found low resolution using ITS rDNA and PKS as genetic markers. These authors proposed two hypotheses to explain this phenomenon: 1) recent gene flow among members of the *C. gracilis* group; or 2) recent speciation of the taxa in the group. The ILD test, which detects heterogeneity among different gene partitions, revealed significant differences in most comparisons (in spite of having eliminated the incongruent samples based

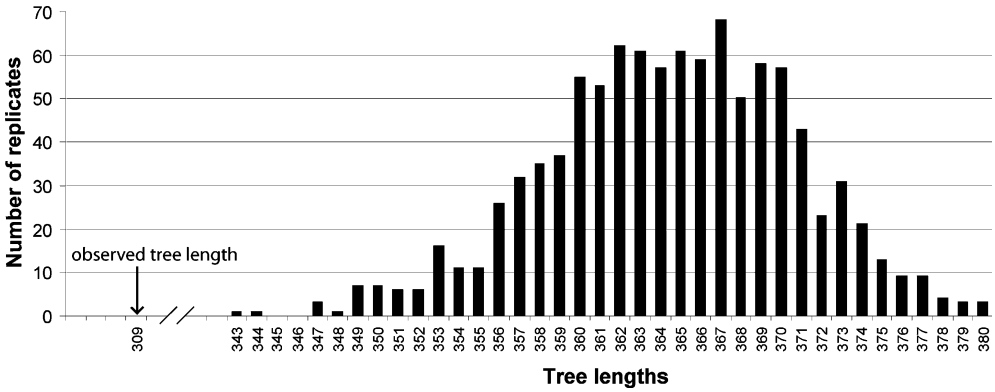

Fig. 3 Results of PTLPT analysis, actual tree length compared to the tree lengths for 1,000 artificially recombined

Table 4 Homoplasy measures of individual characters in the *C. gracilis* group

Character	CI	RI
Atranorin	0.25	0.667
Scyphous podetia	0.111	0.111
Corticate podetia	0.5	0.9
Presence of soralia	0.333	0.333
Squamules on podetia	0.25	0.5
Lateral perforation on podetia	0.25	0.4
Podetial wall thickness	0.143	0.25
White medullary spots	0.333	0.333
Podetial base color	0.4	0.4
Cup width	0.5	0.0

on the Lutzoni et al. (2004) method). The incongruence among loci can be attributed to either recombination, gene flow or incomplete lineage sorting. The two tests used to determine the existence of interlocus recombination, namely association index test and PTLPT test, did not find evidence for recombination. This is consistent with the hypothesis that differences among loci are due to incomplete lineage sorting, according to Fontaine et al. (2010). This hypothesis is also supported by the low number of unique fixed polymorphisms in the clades rendered by the combined analyses (Table 5). However, most of the intralocus recombination analyses did not find recombination signals, but the PHI test did find recombination in the ITS rDNA and *RPB2* data sets. We interpret these as sporadic recombination events. In other groups of lichenized fungi these sporadic recombination events have also been found (Nelsen and Gargas 2009). Therefore, our results indicate that, during the speciation processes in the

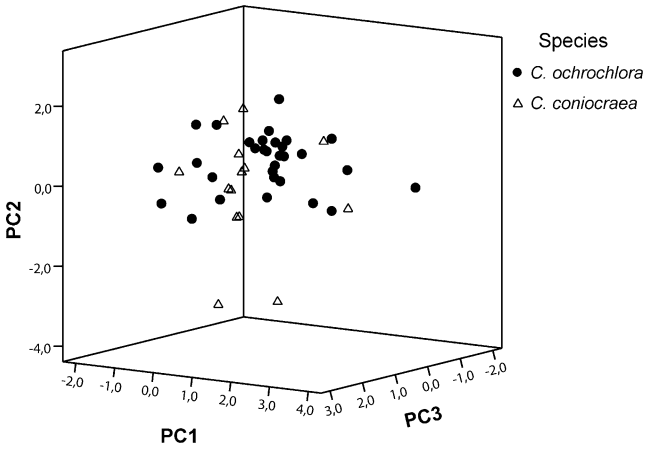

Fig. 4 Principal component analysis (PCA) based on seven morphological and anatomical variables of *C. coniocraea* and *C. ochrochlora*

C. gracilis group, both processes (gene flow and recent speciation) discussed by Fontaine et al. (2010) shaped the phylogeny in these lichenized fungi.

The phylogenetic analyses based on three or four loci did not separate *C. coniocraea* and *C. ochrochlora* into monophyletic groups, agreeing with the suggestion of them being synonymous (Wirth 1995; Thomson 2003). Additionally, we tested using the SH and ELW tests whether our data set is sufficient to reject the monophyly of these two species. Both tests significantly rejected monophyly of *C. coniocraea* and *C. ochrochlora.* Based on morphology, most authors consider them as distinct species, although it is often mentioned that they are difficult to distinguish (Brodo et al. 2001; Awasthi and Ahti 2007; Burgaz and Ahti 2009; James 2009; Fontaine et al. 2010). The morphological differences used to distinguish the two species are as follows: 1) the podetium basal cortex is longer and thicker in *C. ochrochlora*; 2) the presence of discrete soralia between cortex patches in *C. ochrochlora,* while *C. coniocraea* has diffuse soralia; 3) soredia are larger in *C. ochrochlora* than in *C. coniocraea*; 4) presence of broad cups in *C. ochrochlora*, while these are rare in *C. coniocraea*, and narrow when present. Further, in *C. coniocraea* the primary thallus squamules show deep incisions, while in *C. ochrochlora* the squamules are in general undivided (Hammer 1991, 1993, 1995; Ahti 2000; Ahti and Hammer 2002). However, the PCA analysis does not show different groups among the specimens of *C. coniocraea* and *C. ochrochlora*. As for geographical distribution, some differences have been reported between both taxa, *C. ochrochlora* basically having a cosmopolitan distribution, whereas *C. coniocraea* is restricted to the Northern Hemisphere (Ahti 1980b; Ahti 2000). However, Schwerdtner and Cordes (1992), consider that the features which distinguish *C. coniocraea* and *C. ochrochlora* are morphological adaptations to different environmental conditions of a single species. Our results support this interpretation.

The only sample of *C. cornuta* subsp. *groenlandica* included in this study clustered within the clade including *C. coniocraea* and *C. ochrochlora.* This subspecies is morphologically similar to *C. ochrochlora* (Ahti 1980a; Hammer and Ahti 1990; Brodo and Ahti 1996; Brodo et al. 2001). The subspecies, however, is distinguished from *C. ochrochlora* in having a thin stereome, which is thick and hard in *C. ochrochlora* (Ahti 1980b); more frequent and wide cups in *C. cornuta* subsp. *groenlandica* than in *C. ochrochlora;* and the podetia color. While *C. ochrochlora* varies from yellowish green to greyish, the podetia tend to be more brownish in *C. cornuta* subsp. *groenlandica* (Brodo and Ahti 1996). SH and ELW tests rejected the hypothesis that *C. cornuta* subsp. *groenlandica* forms a monophyletic group with *C.*

Table 5 Unique fixed polymorphisms in the highly supported clades resulting from the combined matrix analyses

Samples	RPB2					ITS				IGS							
	9	141	237	343	804	33	429	447	466	158	207	209	226	267	289	302	304
C. ochrochlora 1	T	G	C	C	A	C	A	T	C	T	T	C	C	T	T	G	C
C. ochrochlora 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C. ochrochlora 3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C. ochrochlora 4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C. coniocraea 1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T
C. coniocraea 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C. coniocraea 3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C. coniocraea 4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C. cornuta subsp. *groenlandica*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C. ecmocyna 2	C	C	T	T	G	.	.	.	.	C	C	T	T	.	.	.	G
C. ecmocyna 3	C	C	T	T	G	.	.	.	.	C	C	T	T	.	.	.	G
C. ecmocyna 1	C	C	T	T	G	.	.	.	.	C	C	T	T	.	.	.	G
C. cornuta subsp. *cornuta* 1	C	C	T	T	G	T	.	C	T	.	.	.	.	.	.	T	.
C. cornuta subsp. *cornuta* 2	C	C	T	T	G	T	.	C	T	.	.	.	.	.	.	T	.
C. gracilis subsp. *vulnerata* 1	N	C	T	T	G	.	C	.	.	.	.	.	.	C	C	.	.
C. gracilis subsp. *vulnerata* 2	C	C	T	T	G	.	C	.	.	.	.	.	.	C	C	.	.
C. gracilis subsp. *elongata* 1	C	C	T	T	G	.	.	.	.	.	.	.	.	.	.	.	.
C. gracilis subsp. *elongata* 2	C	C	T	T	G	.	.	.	.	.	.	.	.	.	.	.	.
C. gracilis subsp. *turbinata* 1	C	C	T	T	G	.	.	.	.	.	.	.	.	.	.	.	.
C. gracilis subsp. *turbinata* 2	C	C	T	T	G	.	.	.	.	.	.	.	.	.	.	.	T
C. gracilis subsp. *gracilis* 1	C	C	T	T	G	.	.	.	.	.	.	.	.	.	.	.	T
C. gracilis subsp. *gracilis* 2	C	C	T	T	G	.	.	.	.	.	.	.	.	.	.	.	.
C. gracilis subsp. *gracilis* 3	C	C	T	T	G	.	.	.	.	.	.	.	.	.	.	.	.
C. gracilis subsp. *gracilis* 4	C	C	T	T	G	.	.	.	.	.	.	.	.	.	.	.	.
C. gracilis subsp. *gracilis* 5	C	C	T	T	G	.	.	.	.	.	.	.	.	.	.	.	T
C. macroceras 1	C	C	T	T	G	.	.	.	.	.	.	.	.	.	.	.	.

cornuta subsp. *cornuta*. Consequently, we consider *C. cornuta* subsp. *groenlandica* together with *C. coniocraea* and *C. ochrochlora* as a single species. The oldest available name for this taxon is *C. coniocraea* (Ahti and DePriest 2005).

The specimens of *C. ecmocyna* form a monophyletic clade strongly supported in MP and ML analyses, but in the Bayesian analysis this clade lacks support (pp=0.72). The three specimens included in the combined analyses belong to *C. ecmocyna* subsp. *ecmocyna*. This taxon is characterized by glaucous podetia with a yellow base, and by containing fumarprotocetraric acid and atranorin (Dahl 1950; Ahti 1980a). In spite of its large morphological variability, which caused the description of numerous forms and subspecies (Thomson 1968; Ahti 1980a; Brodo and Ahti 1996), its species rank has not been questioned.

The two specimens of *C. cornuta* subsp. *cornuta* form a monophyletic group, with high support in all the three analyses. In previous studies based on single locus analyses of ITS rDNA and PKS sequences, this taxon was not monophyletic (Fontaine et al. 2010). Morphologically it is similar to *C. ochrochlora,* from which it can be readily distinguished by having longer, brownish and generally unbranched podetia, and soredia that are restricted to the podetial ends or upper half of the podetium (Ahti 1980a; Stenroos et al. 1992; James 2009). The SH and EWL tests rejected the hypothesis that this taxon were closely related to the clade consisting of *C. coniocraea* and *C. ochrochlora*.

In the phylogenetic analyses, *C. gracilis* s.l. is not resolved as a distinct entity. As regards to the infraspecific taxa of *C. gracilis,* only *C. gracilis* subsp. *vulnerata* forms a monophyletic clade; the remaining subspecies are polyphyletic. In addition, the alternative hypothesis tests rejected the monophyly of *C. gracilis* subsp. *gracilis* and *C. gracilis* subsp. *turbinata*. The monophyly of *C. gracilis* subsp. *elongata* was rejected only by the ELW test. These

results agree with those found by Fontaine et al. (2010). The two samples of *C. gracilis* subsp. *vulnerata* form a monophyletic clade, but this relationship is only supported in the Bayesian analysis. This subspecies occurs in North America and Eastern Asia (Ahti 1980a). It is characterized by pale green podetia with a dark base and abundant lateral perforations (Ahti 1980a; Brodo and Ahti 1996). It is morphologically similar to *C. maxima,* which, however, seldom has perforations and has yellowish podetial bases. The available data are insufficient to draw any taxonomical conclusion in this case; this would require a wider study with additional samples.

Conclusions

The large amount of taxonomic uncertainty in the *Cladonia gracilis* group is due to a high amount of homoplasy in the morphological characters. Characters such as the presence of a black basal podetial zone, the presence of white medullary spots on the podetia, the presence of atranorin and the presence of lateral perforations are inconstant within several taxa, yielding low CI and RI values (Table 4). The variable nature of these characters can indicate that they are influenced by environmental conditions or they can be different stages of development. An example is the presence of white medullary spots (that appear in *C. ecmocyna* and *C. macroceras*) on the podetia in *Cladonia subrangiformis* or *C. rangiformis,* to which some authors attribute a scarce taxonomical value, deeming that these spots could be a response to unfavorable conditions (Sandstede 1931) or the accumulation of calcium oxalate in calcareous habitats (James 2009). Another example is the presence of discrete soralia in *C. ochrochlora* which Hammer (1993) considers a stage development. The presence of scyphi and their morphology (especially their width) are the most important characters to distinguish the intraspecific taxa in *C. gracilis.* However, they are strongly homoplasious characters. The development of scyphose or subulate podetia is a capacity present in all the taxa within the *C. gracilis* group, with the exception of *C. gracilis* subsp. *turbinata,* which always and exclusively develops podetia with scyphi (Ahti 1980a).

The morphological character homoplasy in the *Cladonia gracilis* group is not unexpected, since Stenroos et al. (2002) found that most sections in the genus *Cladonia* were circumscribed based on homoplasious characters. Within the genus, the presence of atranorin, the presence of scyphi or soredia are homoplasious characters. Possibly, the phenotypical plasticity of many characters has not been well documented; as a result, the taxonomical value of many characters remains uncertain.

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Appendix 1

Table 6 Character matrix

Samples	1	2	3	4	5	6	7	8	9	10
C. ochrochlora 1	0	0	0	1	0	1	1	0	0	-
C. ochrochlora 2	0	0	0	1	0	1	1	0	0	-
C. ochrochlora 3	0	0	0	1	0	0	1	0	0	-
C. ochrochlora 4	0	0	0	1	0	0	1	0	0	-
C. coniocraea 1	0	0	0	0	0	0	1	0	0	-
C. coniocraea 2	0	0	0	0	0	0	1	0	0	-
C. coniocraea 3	0	0	0	0	0	0	1	0	0	-
C. coniocraea 4	0	0	0	0	0	0	0	0	0	-
C. cornuta subsp. *groenlandica*	0	1	0	0	0	0	1	0	0	0
C. ecmocyna 2	1	0	1	0	1	0	1	1	2	-
C. ecmocyna 3	1	0	1	0	1	0	1	1	2	-
C. ecmocyna 1	1	0	1	0	0	0	1	0	2	-
C. cornuta subsp. *cornuta* 1	0	0	0	0	0	0	1	0	0	-
C. cornuta subsp. *cornuta* 2	0	0	0	0	0	0	1	0	0	-
C. gracilis subsp. *vulnerata* 1	0	0	1	0	0	1	0	0	0	-
C. gracilis subsp. *vulnerata* 2	0	1	1	0	0	1	0	0	0	0
C. gracilis subsp. *elongata* 1	1	1	1	0	0	0	0	0	1	0
C. gracilis subsp. *elongata* 2	0	0	1	0	1	1	0	0	0	-
C. gracilis subsp. *turbinata* 1	0	1	1	0	0	0	0	0	0	1
C. gracilis subsp. *turbinata* 2	0	1	1	0	0	1	1	0	1	1
C. gracilis subsp. *gracilis* 1	1	0	1	0	0	0	0	0	0	-
C. gracilis subsp. *gracilis* 2	0	1	1	0	0	0	1	0	0	0
C. gracilis subsp. *gracilis* 3	1	1	1	0	0	0	0	0	0	0
C. gracilis subsp. *gracilis* 4	0	1	1	0	0	0	0	0	0	0
C. gracilis subsp. *gracilis* 5	0	1	1	0	0	0	1	0	0	0

Table 6 (continued)

Samples	1	2	3	4	5	6	7	8	9	10
C. macroceras 1	1	0	1	0	1	0	1	1	0	-
C. rangiformis 1	1	0	1	0	1	0	?	?	0	-
C. rangiformis 1	1	0	1	0	1	0	?	?	0	-
C. rangiformis 1	1	0	1	0	1	0	?	?	0	-
C. thomsonii 1	1	1	1	0	0	-	?	1	1	-

1) Atranorin: 0 = absent, 1 = present.

2) Morphology of the podetia: 0 = subulate podetia, 1 = scyphous podetia.

3) Corticate podetia: 0 = partly corticate and partly sorediate, 1 = completely corticate.

4) Soralia: 0 = absent, 1 = present.

5) Squamules on podetia: 0 = absent, 1 = present.

6) Lateral perforations on the podetia: 0 = absent, 1 = present.

7) Podetial wall thickness: 0<200 μm, 1>200 μm.

8) Podetia with white medullary spots: 0 = absent, 1 = present

9) Podetial base color: 0 = the same color of the rest of podetia, 1 = black, 2 = yellow.

10) Scyphi with: 0<5 mm of diameter, 1≥5 mm.

Appendix 2. Incongruent samples excluded to the analyses

Cladonia cornuta subsp. *cornuta*. Spain, Soria, *A. R. Burgaz* (MACB 94344). JN811404, JN811433, JN811371.

Cladonia cornuta subsp. *cornuta*. Canada, Newfoundland, *J. C. Lendemer & A. Moroz* (H). JN811403, JN811434, JN811371.

Cladonia gracilis subsp. *elongata*. Chile, Región XII, Magallanes y Antártica Chilena, *A. R. Burgaz* (MACB 91963). JN811405, JN811435, JN811372.

Cladonia gracilis subsp. *gracilis*. Finland, Tavastia Proper, *H. Väre* (H). JN811406, JN811432, JN811370.

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VI

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Cladonia conista and *C. humilis* (Cladoniaceae) are different species

Raquel PINO-BODAS[1], Teuvo AHTI[2], Soili STENROOS[2], María P. MARTÍN[3] & Ana R. BURGAZ[1]

1 Departamento Biología Vegetal 1, Facultad de Biología, Universidad Complutense de Madrid, E-28040 Madrid, Spain; e-mail: rpino@bio.ucm.es, arburgaz@bio.ucm.es
2 Botanical Museum, Finnish Museum of Natural History, P.O. Box 7, FI-00014 University of Helsinki, Finland; e-mail: teuvo.ahti@helsinki.fi, soili.stenroos@helsinki.fi
3 Departamento de Micología, Real Jardín Botánico, CSIC, E-28014 Madrid, Spain; e-mail: maripaz@rjb.csic.es
Corresponding author: Raquel Pino-Bodas; e-mail: rpino@bio.ucm.es

Abstract. *Cladonia conista* and *C. humilis* have primarily been delimited on the basis of their secondary metabolites, i.e. presence of bourgeanic and fumarprotocetraric acid in the former and atranorin and fumarprotocetraric acid in the latter. In this study, sequences from ITS nrDNA, *rpb2* and *ef1α* regions were obtained for the purpose of assessing the taxonomic status of these two chemotypes. Phylogenetic analyses yielded two lineages corresponding with the chemotypes. Morphological analyses only revealed subtle features for distinguishing the lineages. In addition, our study shows that the taxa differ in their geographical distribution: *C. humilis* is predominant on the Pacific coast of America and in Central and South Europe, while *C. conista* is predominant on the Atlantic coast of America and in northern regions of Europe. Therefore *C. conista* (syn. *C. innominata*) and *C. humilis* are recognized as distinct species.

Key words: lichens, taxonomy, cryptic species, species delimitation, *Cladonia*.

Introduction

Cladonia conista (Nyl.) Robbins and *C. humilis* (With.) J. R. Laundon are putative species classified in the genus *Cladonia*, section *Cladonia* (AHTI 2000) or supergroup *Cladonia* (STENROOS et al. 2002). They are characterized by: short-stalked podetia with broad scyphi; the podetial surface is corticated on the stalk, often up to the scyphal margin; the upper podetial surface is farinose sorediate, and the primary thallus is persistent, with well developed squamules. They have usually been regarded as members of the aggregates of *Cladonia chlorophaea* (Flörke ex Sommerf.) Spreng. or *C. fimbriata* (L.) Fr. since their first characterization by ASAHINA (1941, as *C. conista* and *C. conistea*) and EVANS (1944). Both *C. conista* and *C. humilis* are widely distributed: they have been reported from Eurasia (POELT 1962, AHTI 1966, LEUCKERT & POELT 1970, SIPMAN 1973, TØNSBERG 1979, AHTI et al. 1996, AWASTHI & AHTI 2007, BURGAZ & AHTI 2009), North America (EVANS 1930, AHTI & HAMMER 2002, LENDEMER

2008), South America (FERRARO & AHTI 1987, STENROOS et al. 1992, AHTI 2000) and Australasia (MARTIN 1958, ARCHER 1992).
These two taxa can basically be distinguished by their secondary metabolic substances. *C. humilis* produces fumarprotocetraric acid and atranorin (K+ yellow), while *C. conista* produces fumarprotocetraric and bourgeanic acids (K–). On the other hand, the morphological differences between them are only subtle, and most authors have considered the two taxa as morphologically indistinguishable (LAUNDON 1984, AHTI et al. 1996, AHTI 2000). The chemical differences themselves do not reflect systematic relationships (CULBERSON 1986). In fact, there are quite a number of species chemically variable, many within the genus *Cladonia.* The molecular data has recognized *C. arbuscula* (Wallroth) Flotow (PIERCEY-NORMORE et al. 2010) to be a single chemically variable species; the same was proved for *C. subturgida* Samp. (PINO-BODAS et al. 2011). For all these reasons, the taxonomic status of *C. humilis* and *C. conista* has been controversial. For some authors, they are mere chemotypes of a single species (JAMES 2009), while others, on the grounds of the different biogenetic pathway of bourgeanic acid and atranorin, consider that the chemotypes should be accorded a taxonomic status. Some authors treated them as varieties (ARCHER 1989). HOLIEN & TØNSBERG (1985) treated *C. conista* as a species independent from *C. humilis* arguing that its podetia are usually taller than those of *C. humilis;* in addition, *C. conista* is mainly distributed in continental areas, while *C. humilis* has rather an oceanic distribution in Norway.
The aim of this work is to clarify whether *C. conista* and *C. humilis* are two different species, or two chemotypes of the same species, using for that end a molecular phylogeny based on three genetic markers. We will establish too, by means of ITS nrDNA sequences, the phylogenetic relations of *C. conista* and *C. humilis* to other species of *Cladonia*.

Materials and Methods

Lichen material

In total, 296 specimens from the herbaria MACB and H (including a paratype of *Cladonia humilis* var. *bourgeanica*) were closely studied. The material originated from Australia, Chile, Croatia, Finland, Germany, Iceland, India, Japan, Morocco, the Netherlands, Poland, Portugal, Russia, Spain, Turkey, United Kingdom and USA. Specimens of both chemotypes from Europe and North America were selected for molecular study (Table 1). In accordance with STENROOS et al. (2002), *C. rangiformis* was used as an outgroup.

DNA extraction, amplification and sequencing

Total DNA was extracted using DNeasy Plant Mini Kit (QUIAGEN, Germany) following the manufacturer's instructions. The DNA was dissolved in 200 µl of buffer included in the kit. Three genetic regions were selected: ITS nrDNA, *rpb2* partial gene and *ef1α* partial gene. The primers used to amplify the nuclear ITS nrDNA were ITS1F (GARDES & BRUNS 1993) and ITS4 (WHITE et al. 1990), alternatively 1780-5'F/LSU0012 (PIERCEY-NORMORE & DEPRIEST 2001). The *rpb2* partial gene was amplified using nested PCR. The first PCR was performed

Table 1. List of specimens included in molecular analyses with GenBank accession numbers.

Taxa	Locality and collection	ITS	*rpb2*	*ef1α*
C. humilis 1	Portugal, Trás-os-Montes, *A. R. Burgaz* (MACB 92885)	JF926625	JF926584	JF926597
C. humilis 2	Spain, Toledo, *A. R. Burgaz* (MACB 92807)	JF926622	JF926585	JF926598
C. humilis 3	Portugal, Baixo Alentejo, *A. R. Burgaz* (MACB 97326)	JF926626	JF926587	JF926599
C. humilis 4	Portugal, Estremadura, *A. R. Burgaz* (MACB 92818)	JF926627	JF926583	JF926600
C. humilis 5	Spain, Madrid, *A. R. Burgaz* (MACB 95913)	JF926614	JF926575	JF926601
C. humilis 6	Spain, Gerona, *A. R. Burgaz* (MACB 95931)	JF926615	JF926576	JF926602
C. humilis 7	Spain, Mallorca, *A. R. Burgaz* (MACB 92803)	JF926628	JF926581	JF926603
C. humilis 8	Portugal, Madeira, *P. Alanko* (H)	JF926616	JF926582	JF926604
C. humilis 9	Turkey, Trabzon, *K. Yazici* (H)	JF926617	JF926577	JF926605
C. humilis 10	USA, California, *K. Knudsen* (H)	JF926618	JF926578	JF926606
C. humilis 11	USA, California, *T. Ahti* (H)	JF926620	JF926579	JF926607
C. humilis 12	Croatia, Dubrovnik-Neretva, *A. R. Burgaz* (MACB 101103)	JF926621	JF926580	JF926608
C. humilis 13	USA, California, *T. Ahti & L. St. Clair* (H)	JF926624	JF926574	JF926610
C. humilis 14	USA, California, *T. Ahti & L. St. Clair* (H)	JF926623	JF926586	JF926609
C. conista 1	Russia, Kursk Region, *N. T. Zolotuchin* (H)	JF926633	JF926568	JF926590
C. conista 2	USA, Connecticut, *J. C. Lendemer* et al. (H)	JF926634	JF926569	JF926591
C. conista 3	USA, New Jersey, *J. C. Lendemer* et al. (H)	JF926635	JF926570	JF926592
C. conista 4	USA, Pennsylvania, *J. C. Lendemer* et al. (H)	JF926636	JF926571	JF926593
C. conista 5	Spain, Castellón, *A. R. Burgaz* (MACB 97591)	JF926612	JF926566	JF926588
C. conista 6	Spain, Huesca, *A. R. Burgaz* (MACB 92796)	JF926613	JF926567	JF926589
C. conista 7	Spain, Orense, *A. R. Burgaz* (MACB 98080)	JF926630	—	JF926596
C. conista 8	Chile, Region XII, *A. R. Burgaz* (MACB 92033)	JF926631	—	—
C. conista 9	Finland, Laatokan Karjala, *T. Ahti* (H)	JF926629	JF926572	—
C. conista 10	Finland, Etelä-Häme, *V. Haikonen* (H)	JF926632	—	JF926594
C. conista 11	Russia, Sakha Republic, *T. Ahti* (H)	JF926619	JF926573	JF926595
C. rangiformis	Spain, Menorca, *A. R. Burgaz* (MACB 96193)	JF288804	JF288839	JF926611

with the primer pair RPB2-5F/RPB2-7cR (LIU et al. 1999); 1 µl of the first amplification served as DNA template for a second reaction using the primers RPB2dRaq/RPB2rRaq (PINO-BODAS et al. 2010). The primers used to amplify *ef1α* partial gene were CLEF-3F/CLEF-3R (YAHR et al. 2006). The amplification program for ITS nrDNA was: 94ºC for 5 min; 5 cycles of 94ºC for 30 s, 54ºC for 30 s and 72ºC for 1 min; and 33 cycles of 94ºC for 30 s, 48ºC for 30 s and 72ºC for 1 min; with a final extension at 72ºC for 10 min. The amplification program

for *rpb2* was: 94ºC for 5 min; 40 cycles of 95ºC for 30 s, 52ºC for 30 s and 72ºC for 2 min; with a final extension at 72ºC for 10 min. The amplification program for *ef1α* was: 94ºC for 5 min; 35 cycles of 95ºC for 30s, 55ºC for 30s and 72ºC for 1 min; with a final extension at 72ºC for 10 min. PCRs were carried out with Ready-to-Go-PCR Beads (GE Healthcare Life Sciences, UK). Amplifications were prepared for a 25 µl final volume. PCR was performed using the Mastercycler ep Gradient S (Eppendorf). The PCR products were purified using the QIAquick Gel Extraction Kit (QIAGEN). The sequencing reactions were performed in Macrogen (South Korea) sequencing service (www.macrogen.com). Two samples of ITS nrDNA and two of *ef1α* were cloned into pGEM T (Promega). Three clones of each sample were sequenced. The sequences of the different clones were identical and so only one clone was included in phylogenetical analyses.

Sequence alignments and phylogenetic analysis

The sequences were edited and assembled using Sequencher 3.1 (Gene Codes Corporation Inc., Ann Arbor, Michigan, USA). They were manually aligned with SE-AL v2.0a11 (RAMBAUT 1996). The transitions and transversions were considered for aligning the sequences. The ambiguous positions were removed. Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian analyses were performed. The statistical incongruity among the different regions was tested according LUTZONI et al. (2004). Bootstrap analyses were done for every region separately. Every clade with more than 75% of bootstrap was examined to see if any conflict existed between the loci. The phylogenetic analyses were performed using two different matrices. The first, with sequences of ITS nrDNA, *rpb2* and *ef1α* from 25 specimens, was constructed for molecular characterization of *C. humilis* and *C. conista*. A second matrix of ITS nrDNA with 76 sequences (52 of which were downloaded from GeneBank) was constructed to infer the phylogenetic relationships of *C. humilis* and *C. conista* with other members of the supergroup *Cladonia*. Samples with short sequences of ITS rDNA (*C. conista* 7 and *C. conista* 11) were excluded from this dataset because they introduced noise into the analyses.

MP analyses were conducted with PAUP* version 4.0b10 (SWOFFORD 2002). Heuristic search with 1000 replicates and TBR Branch-swapping option were used in the analyses. Bootstrap analyses were performed with 1000 replicates, using the heuristic option. ML analyses were implemented with Tree-Puzzle 5.2 (SCHMIDT et al. 2002) assuming GTR+I+G model. MrModeltest (NYLANDER 2004) was used for selecting the best evolution model by AIC criterion. SYM+G model was selected for ITS nrDNA; SYM+I model for *rpb2;* HKY+I for *ef1α.* SYM+I+G model was selected for the second dataset. A Bayesian approach was carried out by MrBayes 3.1 (HUELSENBECK & RONQUIST 2001) using the above models. The first dataset was divided in seven parts (ITS nrDNA, each of the three codon positions of *rpb2* and each of the three codon positions of *ef1α)*. The posterior probabilities were approximated by sampling trees using Markov Chain Monte Carlo (MCMC). The posterior probabilities of each branch were calculated by counting the frequency of trees that were visited during the course of the MCMC analysis. Model parameters were estimated in each analysis for

10.000.000 generations sampled in 4 simultaneous chains, and every 1000th was saved into a file. Tracer v. 1.0 (http://evolve.zoo.ox.ac.uk/software.html?id=tracer) and AWTY (NYLANDER et al. 2008) were used to determine when the chains reached the stationary stage. Then, the initial 1000 trees were discarded as burn-in. Using the "sumt" command of MrBayes, the 50% majority-rule consensus tree was calculated from 18000 trees sampled after reaching likelihood convergence to calculate the posterior probabilities of the tree nodes.

Morphological and chemical studies

For morphological analyses, measurements of podetium length, podetial stalk length, scyphi width, extension of cortex on podetia, were taken. Microscopic measurements of the podetial wall thickness and soredium size (and also partial measurements of the layer, cortex, medulla and stereome thickness) were made at x400, using an Olympus CX41 microscope. The hand made sections were mounted in distilled water. For photographs, an Olympus SZX9 dissecting microscope and a DP11 (Olympus) camera were used. The statistical analyses were carried out with STATGRAPHICS 5.1 program. The Kolmogorov-Simonov test was used to check normality, while Levence statistic was employed to check the homogeneous variance. The variables which fulfilled both conditions were analyzed with ANOVA and those that did not fulfill them were analyzed with the nonparametric Kruskal-Wallis test.

Lichen compounds were identified using thin layer chromatography (TLC) in solvents A and B according to the standardized protocols of WHITE & JAMES (1985). The colour reaction of the thallus with K test was checked in 63 specimens.

Results

Phylogenetic analyses

Seventy one new sequences were generated in this study, 25 of ITS nrDNA, 24 of *ef1α* and 22 of *rpb2*. The independent analyses of each region for the first dataset (which contains only *C. conista* and *C. humilis* samples) did not show any conflict, and the data of the three genetic regions were combined. The ambiguous positions were excluded (19 sites in ITS nrDNA matrix) and the dataset was composed of 2174 characters (639 of ITS nrDNA, 635 of *ef1α* and 900 of *rpb2*). Of these, 1908 were constant and 92 were parsimony informative. Maximum parsimony analysis for the first dataset generated 1000 equally parsimonious trees, 293 step long, CI = 0.9625, RI = 0.9821 and RC = 0.9453. The ML analysis yielded a tree with a likelihood value of Ln = -4905.20 and the likelihood value of Bayesian analysis was LnL = -4823.24. The parameters of Bayesian analysis are shown in Table 2. The trees of MP, ML and Bayesian analyses showed the same topology, whereby only the consensus Bayesian tree is shown (Fig. 1). *C. humilis* and *C. conista* form two monophyletic clades with a high support.

The second dataset (which consist of ITS rDNA sequences of taxa of the supergroup *Cladonia*) contained 567 characters, 117 of them parsimony

informative (Fig 2). MP analysis generated 1000 equally parsimonious trees, 547 steps long, CI = 0.543, RI = 0.770 and RC = 0.4184. The ML analysis yielded a tree with a likelihood value of Ln = - 4372.73 and Bayesian approach Ln = - 3996.61. The likelihood parameters of Bayesian analysis are shown in Table 2. The majority-rule consensus of the Bayesian analysis is reported in Fig. 2. *C. humilis* and *C. conista* cluster into a well-supported clade, close to the taxa of the *C. furcata* (Huds.) Schrad. group and other taxa of the *C. humilis* group, such as the East Asian species *C. kurokawae* Ahti & S. Stenroos and *C. subconistea* Asahina. *C. conista* appears to be more closely related to *C. kurokawae* and *C. subconistea* than *C. humilis*.

Table 2. Parameters of Bayesian analyses [mean value (variance)]

Parameter	Combinated	ITS rDNA
π (A)	0.259 (0.00007)	-
π (C)	0.248 (0.00007)	-
π (G)	0.241 (0.00007)	-
π (T)	0.250 (0.00007)	-
r (A-C)	0.079 (0.00033)	0.063 (0.00014)
r (A-G)	0.228 (0.00087)	0.201 (0.00145)
r (A-T)	0.097 (0.00041)	0.119 (0.00028)
r (C-G)	0.056 (0.00025)	0.547 (0.00008)
r (C-T)	0.453 (0.00127)	0.547 (0.00222)
r (G-T)	0.086 (0.00038)	0.039 (0.00390)
α	91.9515 (3659.2648)	0.238 (0.00390)
κ	3.0041 (0.5925)	-
Pinvar	0.5663 (0.0072)	0.188164 (0.00525)

Morphological and chemical studies

The morphological analyses revealed some phenotypic differences between *C. humilis* and *C. conista* (Table 3, Figs 3–4). *C. conista* has longer podetia, wider scyphi and longer podetial stalks than *C. humilis*. However, the soredia of both taxa are of a similar size. The corticated podetial surface is similar in *C. conista* and *C. humilis*. No significant difference in podetial wall thickness was observed between *C. humilis* and *C. conista*. As can be observed in Figs 3–4, both taxa show great intraspecific variability, and specimens of *C. humilis* are often found with podetia which are morphologically closer to those of *C. conista,* and *vice versa*.

The TLC studies on 251 samples revealed that *C. humilis* contains atranorin and fumarprotocetraric acid. In addition, zeorin, confumarprotocetraric, physodalic and hypoprotocetraric acids were identified in 9, 12, 36 and 54 samples, respectively. In *C. conista,* apart from fumarprotocetraric and bourgeanic acids, confumarprotocetraric acid was found in 6 samples, physodalic acid in 1 sample and hypoprotocetraric acid in 1 sample. AHTI (2000) reported the presence of protocetraric acid as well. The minor compounds zeorin, physodalic and hypoprotocetraric acids are new reports for these species. The K test yielded a dingy yellow-brownish reaction in 42.3% of the specimens, while 57.7% did not show a distinct reaction. In *C. humilis* 75.7% of the specimens reacted bright yellow, 13.5% yellow-brownish, and 10.8% of the reactions were negative.

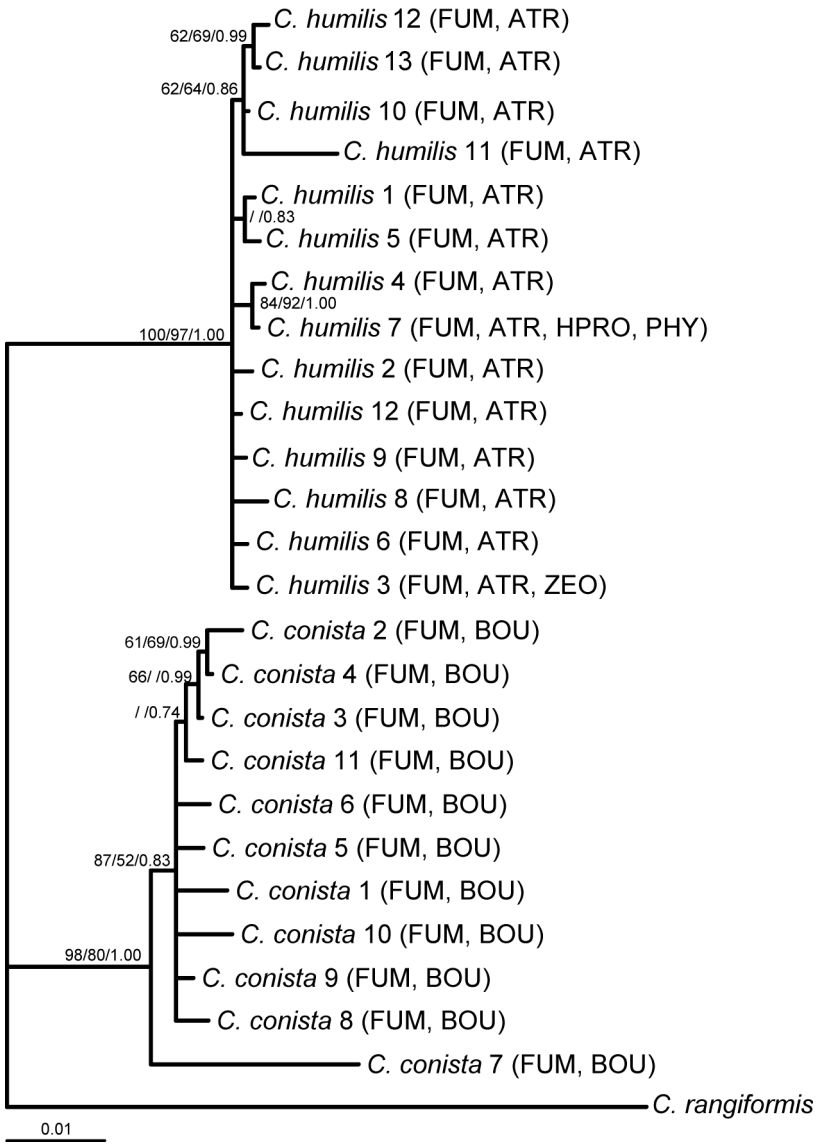

Fig. 1. The 50% majority-rule consensus tree from Bayesian analysis based on ITS nrDNA, *ef1α* and *rpb2*. The bootstrap values of MP and ML analyses and posterior probability of Bayesian analysis are indicated on the branches. ATR = atranorin, BOU = bourgeanic acid, FUM = fumarprotocetraric acid, HPRO = hypoprotocetraric acid, PHY = physodalic acid, and ZEO = zeorin.

Geographical distributions of C. conista and C. humilis

Based on the material revised in numerous herbaria by the author TA during 45 years, or published by authors which he has judged to be obviously reliable, we mapped the distribution of both taxa. The following differences in the distributions can be observed: *C. humilis* occurs in both North and South America, predominantly on the Pacific coast; it is also abundant in Western, Central and South Europe. In Asia, it is known from Nepal, the Pacific coast of China, and Japan. In Australasia, it has been found in South Australia, Tasmania and in the North and South Islands of New Zealand. In Africa, it has been found in the

Canary Islands, Azores, Ethiopia and South Africa. *C. conista,* on the contrary, is distributed on the Atlantic coast of America, but it is also abundant elsewhere in eastern North America. In Europe, *C. conista* is more abundant than *C. humilis* in northern regions; it also occurs in Central and South Europe, where *C. humilis* is more abundant. In Asia, *C. conista* is widespread in Siberia and China, also in Japan and Nepal. In Australasia, it is found in Australia (Victoria, New South Wales and Tasmania) and on the South Island of New Zealand.

Fig. 2. Phylogenetic emplacement of *C. humilis* and *C. conista* in the supergroup *Cladonia* based on ITS nrDNA sequences. The 50% majority-rule consensus tree from Bayesian analysis. Bold branches indicated a support ≥ 70% of Bootstrap and 0.95 of posterior probability.

Discussion

The phylogenetic analyses based on three DNA sequence loci have shown that *C. conista* and *C. humilis* form two monophyletic highly supported independent clades. According to the genealogical concordance phylogenetic species recognition (TAYLOR et al. 2000), these clades represent two phylogenetic species, confirming that the chemical differences found between them indicate a taxonomic distinction, as proposed by other authors (EVANS 1930, ARCHER 1989, LENDEMER 2008).

DOLNIK et al. (2010) stated that the material of *C. conista* and *C. humilis* was different with respect to the ITS nrDNA region. This material was collected in Central Europe. Our results, based on increased sampling (including specimens from North America and Europe), confirm their findings. There is a third chemotype in this group, containing fumarprotocetraric acid, atranorin and bourgeanic acid, i.e. combining the chemistries of *C. humilis* and *C. conista*. This chemotype was found in Argentina (STENROOS et al. 1992, AHTI 2000). We have not dealt with this chemotype in the present study because of the lack of fresh material. Further studies are required to assess the status of the third chemotype.

C. conista and *C. humilis* are not phylogenetically very closely related species, but *C. conista* is close to *C. kurokawae* and *C. subconistea,* a similar result having been attained by STENROOS et al. (2002). *C. kurokawae* and *C. subconistea* can be distinguished from *C. conista* by their secondary metabolites and the size of soredia (AHTI et al. 1996). *Cladonia humilis* is likely to be more closely related to some species of the *C. humilis* group not included in this work. In the latter group, as well as *C. conista*, *C. humilis*, *C. kurokawae* and *C. subconistea,* the species *C. hammeri* Ahti, *C. nashii* Ahti and *C. pulvinella* Hammer are included. This hypothesis will be tested in our future work on the *C. humilis* group.

The taxa studied here were traditionally discussed within the groups of *C. chlorophaea* and *C. pyxidata* (L.) Hoffm. (AHTI 1966, COASSINI-LOKAR et al. 1986, KOWALEWSKA et al. 2008). The reason is that their podetia have a similar phenotypic structure, possessing wide scyphi with soredia, granules or plates in the interior and on the outer surface of the cups. However, the phylogeny published by STENROOS et al. (2002) proved that wide-scyphose species do not form a monophyletic group within *Cladonia.* Even *C. pyxidata* is polyphyletic (STENROOS et al. 2002, KOTELKO & PIERCEY-NORMORE 2010) and this morphospecies is constituted by several phylogenetic species, apparently indistinguishable from a morphological viewpoint. The members of the *C. chlorophaea* group are not very closely related to *C. humilis* or indeed *C. conista* (Fig. 2). The included taxa in the *C. furcata* group (*C. furcata, C. scabriuscula* (Delise) Nyl., *C. farinacea* (Vain.) A. Evans) are morphologically very different from *C. humilis* and *C. conista,* but they are phylogenetically closer to them than those of the *C. chlorophaea* group. A similar result was reported by STENROOS et al. (2002). In the *Caloplaca citrina* group, for instance, the study of the ITS nrDNA region proved that some species, indistinguishable from a morphological standpoint, are not closely related (VONDRÁK et al. 2009). It was recently proved that, within family Cladoniaceae, some species classified in different genera due

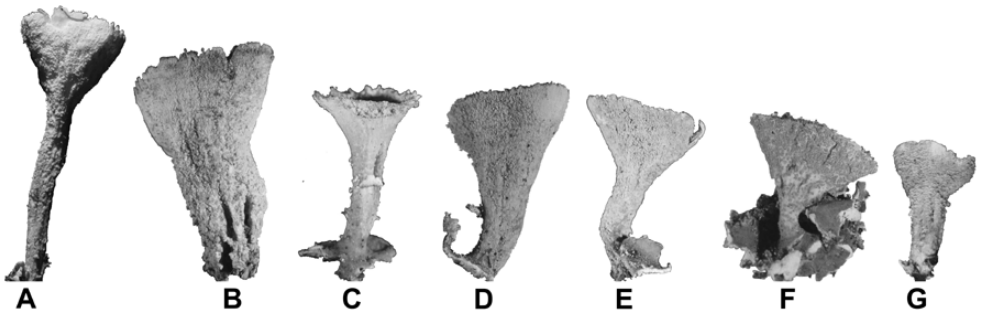

Fig. 3. Morphological variability of *C. conista* A) MACB 98033, B) MACB 92023, C) MACB 98085, D) H (Zolotuchin, 29-IX-2006), E) MACB 92896, F) H (Lendemer, 22-IV-2008), G) MACB 96023.

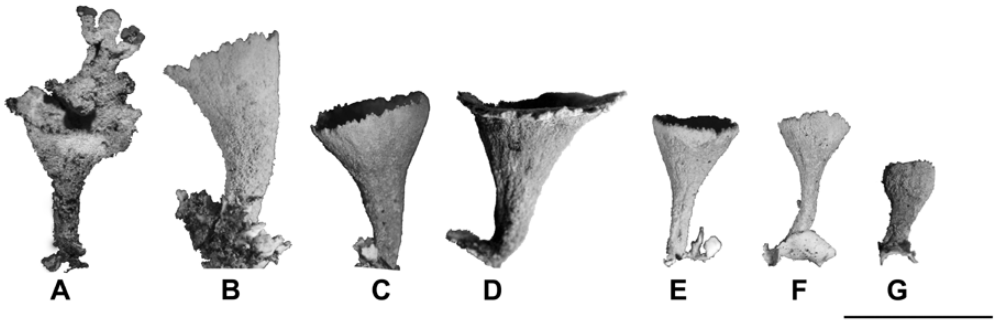

Fig. 4. Morphological variability of *C. humilis* A) MACB 97944, B) MACB 92882, C) MACB 92881 D) H (Ahti 68970), E) MACB 93013, F) MACB 96057, G) MACB 92870.

to their different growth forms (*Cladia, Heterodea* and *Ramalinora*) in fact belong to a single genus (PARNMEN et al. 2010, LUMBSCH et al. 2010).

Cladonia humilis and *C. conista* both have a subcosmopolitan distribution, although some biogeographical differences have been reported. Some authors have pointed out that *C. conista* grows in more continental areas, while *C. humilis* has a rather oceanic distribution (HOLIEN & TØNSBERG 1985, KOWALEWSKA et al. 2008). In the present work, the world distribution maps of both taxa (Fig. 5) are included, showing that their distribution is different.

Very little is known about the different ecological requirements of both taxa. *C. conista* has been collected in exposed sites, such as dunes on acid substrates rich in humus (STENROOS et al. 1992, HOLIEN & TØNSBERG 1985, KOWALEWSKA & SZOK 2004). *C. humilis* also has a preference for weakly acid substrates, and is often found on sandstone, on sandy spots among rocks and, more rarely, on tree stumps (COASSINI-LOKAR et al. 1986, FERRY & PICKERING 1989).

Morphologically, *C. conista* and *C. humilis* are very similar species. In the present study some differences were found, such as the podetium length, as already pointed out by HOLIEN & TØNSBERG (1985). Nevertheless, these morphological differences are very subtle, indicating morphological trends rather than clear discriminating features. On occasion, it is difficult to resolve which taxon we are dealing with based solely on a morphological study. In the early stages of development, the podetia of *C. conista* and *C. humilis* are probably indistinguishable. Therefore, morphological identification should be confirmed by a chemical study.

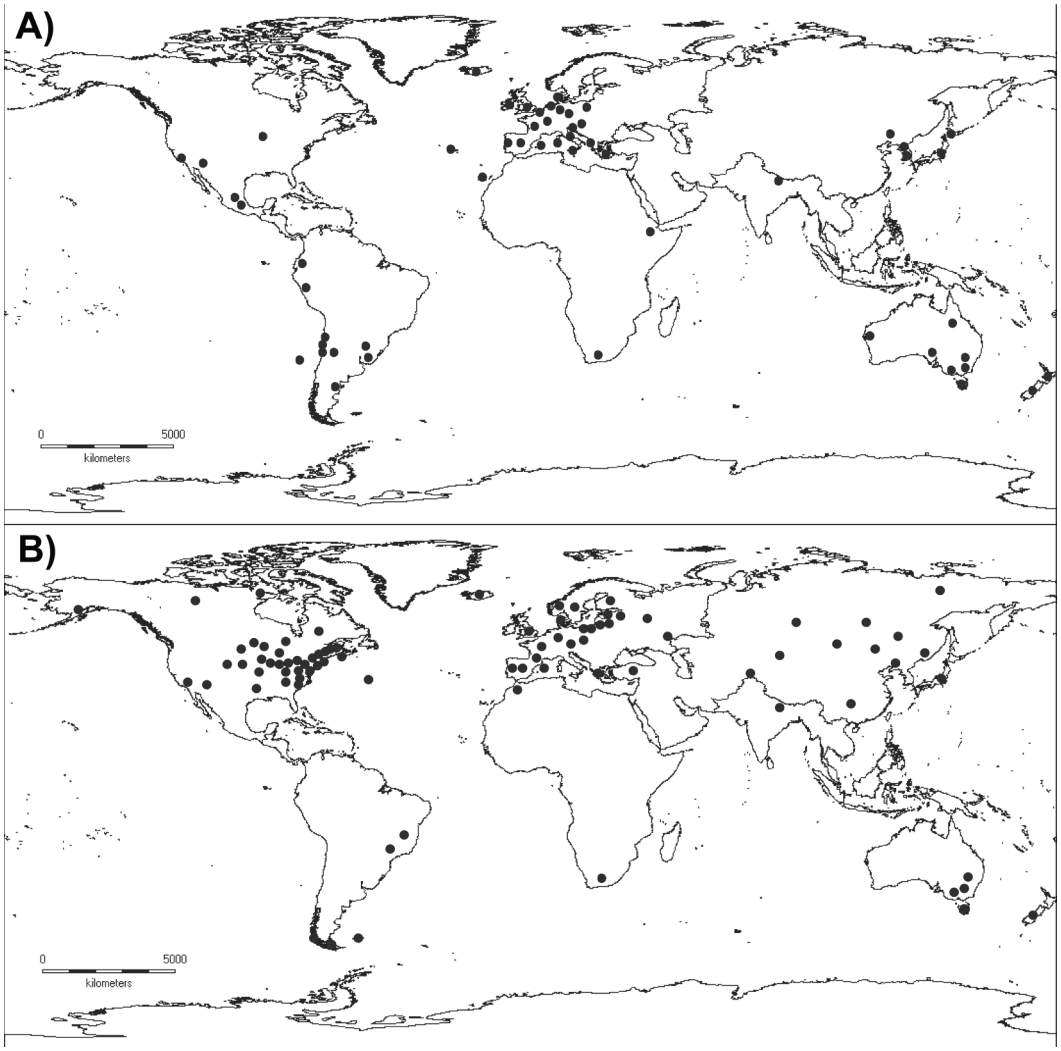

Fig. 5. Distribution maps of A) *C. humilis* and B) *C. conista*.

The K reaction is usually carried out to detect the presence of atranorin. This test was used by EVANS (1930) and HAMMER (1991) to separate *C. conista* from *C. humilis* (‘*C. conistea*’). Our results are similar to these authors’. In a high number of cases, the test yields a brownish-yellow result with *C. conista,* while it yields a bright yellow one with *C. humilis.* EVANS (1930) found that the K reaction becomes brown-blackish in *C. conista* upon drying, but we could not confirm this. We could consider *C. conista* and *C. humilis* as semi-cryptic species, a concept defined by VONDRÁK et al. (2009) to refer to phylogenetic species which are morphologically indistinguishable, but show ecological or distributional differences.

Nomenclature

Cladonia humilis (With.) J. R. Laundon
Lichenologist 16: 220. 1984. – *Lichen humilis* With., Bot. Arr. Veg. Gr. Brit. 721 (1776). – Type: Icon of *Coralloides scyphis humilibus intus fuscis* Dillenius, Hist. Musc. T. 14, f. 11 (1742, ’1741’); England, London, Greenwich, Charlton and Woolwich, Herb.

Dillenius No. 86.11 (OXF, epitype, designated by AHTI, 2000: 122; as ”typotype” by LAUNDON, 1984: 220). Contains atranorin and fumarprotocetraric acid.
Cladonia pyxidata [unranked] *exilis* Hoffm., Deutschl. Fl. 2: 121 (1796). – *Baeomyces pyxidatus* var. *exilis* (Hoffm.) Ach., Methodus 338 (1803). – Type: Homotypic with *Lichen humilis* With. (lectotype designated by LAUNDON 1984: 220).
Cladonia conoidea Ahti, Lichenologist 129 (1980). – Type: England, Isles of Scilly, St. Mary's, Penninis Head, 1979, P. W. James (BM, holotype; H, isotype). Contains atranorin and fumarprotocetraric acid.
Misapplied name: *Cladonia conistea* (Delise) Asahina, J. Jap. Bot. 19: 234 (1943) (see AHTI 1980: 129).

Cladonia conista (Nyl.) Robbins
in Allen, Rhodora 32: 92. 7 May 1930 (antedating Robbins in Evans, Trans. Connecticut Acad. Arts Sci. 30: 472. June 1930). – *Cenomyce fimbriata* var. *conista* Ach., Syn. Lich.: 257 (1814), nom. illeg. – *Cladonia fimbriata* f. *conista* Nyl., Ann. Sci. Nat., Bot., sér. 4, 15: 370 (1861), nom. nov. (Art. 58.1) – Type: “Germania, a Floerke 1” (H-ACH 1705A, lectotype designated by AHTI 1966: 387 and more precisely in AHTI 1980: 129). [Later typification from *“Cladonia conista* A. Evans 1930” – which contains a new description – becoming superfluous]. Contains bourgeanic and fumarprotocetraric acids.
Cladonia conista f. *simplex* Robbins in Evans, Trans. Connecticut Acad. Arts Sci. 30: 473 (1930). – Type: USA, Connecticut, New Haven Co., North Branford, 1927, A. W. Evans & F. A. Musch (US-Evans, lectotype, designated here). Contains bourgeanic and fumarprotocetraric acids.
Cladonia conista f. *centralis* Dix, Bartonia 25: 83 (1949). – Type: USA, Pennsylvania, Berks Co., Scarlett's Mills, 1944, W. L. Dix (PH, holotype).
Cladonia humilis var. *bourgeanica* A.W. Archer, Muelleria 7: 3 (1989). – Type: Australia, New South Wales, Six Foot Track, Binomea Ridge, 2 km N of Jenolan Caves, 1987, A. W. Archer 2086 (MEL, holotype; NSW, isotype). Contains bourgeanic and fumarprotocetraric acids.
Cladonia innominata Lendemer, Mycotaxon 104: 326 (2008). – Type: USA, Connecticut, Litchfield Co., Canaan, W of Sand Rd., 2003, J. Lendemer et al. 1358 in Lendemer, Lich. East. N. Amer. Exs. 142 (NY, holotype; H, isotype). Contains bourgeanic and fumarprotocetraric acids.
The nomenclature of this species has been problematic. As explained by AHTI (1980: 129), *Cenomyce fimbriata* var. *conista* Ach. is a superfluous name because an earlier epithet used as variety, *Baeomyces pyxidatus* var. *exilis* (Hoffm.) Ach. (ACHARIUS 1803), was cited in synonymy. However, due to the fairly recent addition to the Code, Article 7.5, the type of var. *conista* is not automatically that of var. *exilis*. This is because the epithet *exilis* was cited under “a subordinate taxon that did not include the evidently intended type of the illegitimate name”. Therefore the lectotypification by AHTI (1966) is tenable, although, e.g. LAUNDON (1984), AHTI (1993, 2000) and LENDEMER (2008) have not accepted it.

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Multilocus approach to species recognition in the *Cladonia humilis* complex (Cladoniaceae, Ascomycota)

VII

ARTÍCULO VII

Multilocus approach to species recognition in the *Cladonia humilis* complex (Cladoniaceae, Ascomycota)

Raquel Pino-Bodas, Teuvo Ahti, Soili Stenroos, María P. Martín & Ana Rosa Burgaz

American Journal of Botany (2012) En revisión

El complejo de *Cladonia humilis* está formado por un conjunto de taxones caracterizados por tener un talo primario bien desarrollado y podecios escifosos. Las especies de este complejo se distribuyen en las regiones templadas, estando muy representadas en las regiones Mediterráneas tanto de Europa como de Norteamérica. Los taxones que forman este complejo son: *C. conista*, *C. cyathomorpha*, *C. hammeri*, *C. humilis*, *C. kurokawae*, *C. nashii*, *C. pulvinella* y *C. subconistea*. Los caracteres usados para distinguir las especies son el tamaño de los soredios o de los gránulos, la presencia o ausencia de córtex sobre los podecios, y los metabolitos secundarios. En el presente estudio se llevó a cabo un análisis filogenético de este complejo con el objetivo de revisar si los límites entre las especies estaban bien establecidos. Para esta finalidad se investigaron cuatro loci: ITS rDNA, IGS rDNA, *rpb2* y *ef1α*. Los análisis filogenéticos han mostrado que el complejo de *C. humilis* no es monofilético, debido a que *C. nashii* no está estrechamente relacionada con el resto de taxones de este complejo. La diversidad de este complejo se distribuye en siete clados que corresponden a: *C. conista*, *C. cyathomorpha*, *C. hammeri* s.s., *C. humilis* s.l., *C. nashii*, las muestras de *C. kurokawae* y *C. subconistea* y por último un clado formado por especímenes de *C. hammeri* y *Cladonia* sp. Las muestras de *C. hammeri* de Norteamérica son monofiléticas y genéticamente diferentes de las de Europa. La muestras de *C. pulvinella* no formaron un clado monofilético independiente, sino que aparecen agrupadas con las muestras de *C. humilis*. La mayor parte de los linajes filogenéticos están formados por especímenes de más de un quimiótipo, lo que resta valor taxonómico a estos caracteres para discriminar las especies de este complejo.

Multilocus approach to species recognition in the *Cladonia humilis* complex (Cladoniaceae, Ascomycota)

Raquel Pino-Bodas[1], Teuvo Ahti[2], Soili Stenroos[2], María P. Martín[3], Ana R. Burgaz[1]

1 Deptartamento de Biología Vegetal 1, Universidad Complutense de Madrid, Spain; 2 Botanical Museum, Finnish Museum of Natural History, P.O. Box 7, FI-00014 University of Helsinki, Finland; 3 Deptamento de Micología, Real Jardín Botánico, CSIC, Spain

Abstract: The *Cladonia humilis* complex is an assemblage of taxa characterized by a well-developed primary thallus, and rather short and broadly scyphose podetia. In the present study, a phylogenetic analysis of the *Cladonia humilis* complex was carried out in order to elucidate whether the boundaries between the species are properly established. For this purpose, four DNA loci, viz. ITS rDNA, IGS rDNA, *rpb2* and *ef1α* were sequenced. The performed phylogenetic analyses showed that *C. humilis* complex is not monophyletic. *Cladonia nashii* is not closely related to the remaining taxa within the complex. The samples of *C. hammeri* from North America are genetically different from those in Europe. Most of the phylogenetic lineages comprise specimens belonging to more than one chemotype.

Keywords: Species delimitation, lichen, *Cladonia*, secondary metabolites, genealogical sorting index.

Introduction

In recent years much attention has been paid to the problem of species delimitation in different groups of organisms (Göker et al. 2009; Groeneveld et al. 2009; Meudt et al. 2009; Wedin et al. 2009; Carstens & Dewey 2010; Pagès et al. 2010; Gazis et al. 2011; Sakalidis et al. 2011), a task which, in many cases, involves a great challenge. Currently, it is widely accepted that the data from DNA sequences are necessary to inspect the species boundaries and establish which groups need a taxonomical revision (Lauson et al. 2004; Göker et al. 2010; Lumbsch & Leavitt, 2011). In the lichenized fungi the molecular data in many cases have revealed incongruities between phenotypic characters and phylogenetic results (Ott et al. 2004; Otálora et al. 2010; Leavitt et al. 2011); in other cases, totally cryptic species have been detected (Kroken & Taylor 2001; Lumbsch et al. 2008; Vondrák et al. 2009; Molina et al. 2011).

In groups of closely related species, whenever the divergence time is comparatively short with respect to the ancestral population effective size in a certain locus, it is frequent that species do not appear as monophyletic, even if they are reproductively isolated, since monophyly is the final stage in divergence process (Cummings et al. 2008). This phenomenon, known as incomplete lineage sorting, has raised difficulties in establishing limits and relationships among species in numerous groups of organisms (Takahashi et al. 2001; Morando et al. 2004; Heckman et al. 2007; Willyard et al. 2009; Gurushidze et al. 2010). In lichenized fungi, evidence for

incomplete lineage sorting have been found in *Xanthoria* (Lindblom & Ekman 2005), *Peltigera* (Goffinet et al. 2003), *Porpidia* (Buschlom & Muller 2006), *Thamnolia* (Nelsen & Gargas 2009) and *Cladonia* (Myllys et al. 2003; Piercey-Normore et al. 2010), among others. A simple method to assess the degree of genealogic divergence, known as genealogical sorting index (GSI) (Cummings et al. 2008), has recently been developed. This method pursues a quantification of the exclusive ancestry of individuals in user labelled groups on a rooted tree.

Among macrolichens, *Cladonia* is one of the genera with greatest evolutionary success, approaching 500 described species, distributed the world over. It is characterized by a dimorphic thallus composed of a squamulose or crustose primary thallus, that can be persistent or evanescent, and a fruticose secondary thallus, which is morphologically very variable. The morphological characters linked to the secondary thallus, along with the extrolites (secondary metabolites), are the ones primarily used for species differentiation. Based on these characters, the genus *Cladonia* was divided by Ahti (2000) into seven sections (*Ascyphiferae, Cladonia*, *Cocciferae, Helopodium, Perviae, Strepsiles*, and *Unciales*), while *Cladina* was considered to be a different genus. Nevertheless, in the phylogeny proposed by Stenroos et al. (2002), based on the study of ITS rDNA and *β-tubulin* loci, all the sections of the genus turned out to be polyphyletic, while *Cladina* was proved to be a monophyletic group within *Cladonia*. The quoted authors proposed a provisional classification of the genus in three subdivisions and four supergroups. A considerable number of studies on *Cladonia* using molecular data have been carried out in recent years (Printzen & Ekman 2003; Guo & Kashiwadani 2004; Yahr et al. 2006; Beiggi & Piercey-Normore 2007; Parnmen et al. 2008; Robertson & Piercey-Normore 2007; Dolnik et al. 2010; Kotelko & Piercey-Normore 2010; Fontaine et al. 2010; Piercey-Normore et. al. 2010; Pino-Bodas et al. 2010a, 2010b). Many of them had the aim of elucidating the taxonomic value of phenotypic characters to distinguish species. Species delimitation in *Cladonia* is often not easy, owing to the high morphological variability, to the existence of numerous complexes of closely related species, and to the proved fact that many phenotypic characters are homoplastic. Among the homoplastic characters the following ones are examples: presence of soredia, presence of cortex, production of scyphi, open axils, presence of atranorin (Stenroos et al. 2002).

This study focuses on the *Cladonia humilis* complex, which comprises species characterized by a well-developed primary thallus, small-sized, low-growing podetia in general, wide scyphi and soredia or granules on the scyphal surface or inside the scyphi (Pino-Bodas et al. 2012). This complex includes *C. cyathomorpha* Stirt. ex Walt. Watson*, C. humilis* (With.) J. R. Laundon*, C. kurokawae* Ahti & Stenroos*, C. pulvinella* Hammer and *C. subconistea* Asahina (Ahti 2000). Later on two more species were described, *C. hammeri* Ahti and *C. nashii* Ahti (Ahti & Hammer 2002) that morphologically belong to this complex. *Cladonia humilis* was considered by many authors as a species containing two chemotypes (Ahti 2000; James 2009), while in other authors' view each chemotype was a different taxon (Holien & Tønsberg 1985; Archer 1989). A recent phylogenetic work (Pino-Bodas et al. 2012) proved that these two chemotypes are genetically different, whereby two species, *C. humilis* and *C. conista* (Nyl.) Robbins are recognized. Species distinction within the *C. humilis* complex is based on the soredia (or granules) size, on the presence or absence of corticate podetia and on the characteristics of these, as well as on the secondary metabolites. Yet it is often difficult to set the limits of the species; on the other hand the taxonomical value of some of the mentioned characters has

been questioned. For example, Paus et al. (1993) pointed out that soredia size is probably influenced by environmental conditions or it depends on the individual development stage. *Cladonia conista, C. humilis, C. nashii* and *C. subconistea* (Asahina 1941; Evans 1930; Ahti & Hammer 2002) show farinose soredia, while *C. cyathomorpha*, *C. hammeri*, *C. kurokawae* and *C. pulvinella* (Hammer 1991; Ahti et al. 1995; Ahti & Hammer 2002) present granules or schizidia. *Cladonia conista, C. humilis* and *C. subconistea* have a smooth cortex reaching near the scyphal edge, while *C. kurokawae* has a rugose cortex (Ahti et al. 1995), and *C. cyathomorpha* a rugose-areolate one. Further, the cortex of *C. hammeri* in general restricts itself to the podetial base, and can extend to half the podetium, and *C. pulvinella* and *C. nashii* generally lack a cortex. As for secondary metabolites, *C. humilis*, *C. kurokawae* and *C. nashii* contain fumarprotocetraric acid and atranorin; *C. conista,* fumarprotocetraric and bourgeanic acids; *C. pulvinella,* atranorin, fumarprotocetraric and bourgeanic acids, though on occasion atranorin is absent (Ahti 2000; Burgaz & Ahti 2009); *C. hammeri* and *C. cyathomorpha* contain fumarprotocetraric acid and it has been quoted that *C. cyathomorpha* can contain another compound known as CYAT (Jølle 1977), or atranorin (Ahti 1966); *C. subconistea* contains atranorin and psoromic acid (Asahina 1941).

Species diversity in this group is concentrated in warm temperate regions, incl. Mediterranean climatic regions of North America and Eurasia, although some species, such as *C. conista, C. cyathomorpha* and *C. humilis,* have a wider distribution. *Cladonia hammeri* and *C. pulvinella* have been reported in North America (Ahti & Hammer, 2002; Hammer 1995), Europe and Canary Islands (Burgaz & Ahti, 1998 2009; Pérez-Vargas, 2008; Sicilia et al. 2009); *C. nashii* has only been found as yet in North America (Ahti & Hammer 2002).

The purpose of our study is to achieve a phylogenetic reconstruction of the *C. humilis* complex using four nuclear genes and to determine whether the current species delimitation in the complex, based on phenotypical characters, accords with the phylogenetic results. Whenever species turn out to be non-monophyletic, GSI will be applied. We start from the hypothesis that species whose distribution is restricted to Mediterranean climate areas in Europe, on the one hand, and in North America, on the other hand, can be genetically different owing to the great geographic distance that separates them.

Materials and methods

Sampling taxa

Eigthy seven samples of the *C. humilis* complex coming from different regions (Table 1) were selected for phylogenetic analyses. The samples are from the herbaria MACB, H, F, or UC. The species included are *C. conista*, *C. cyathomorpha*, *C. hammeri*, *C. humilis*, *C. kurokawae*, *C. nashii*, *C. pulvinella* and *C. subconistea* plus some samples that by their morphological characters did not satisfactorily correspond to any of the described species; these samples are codified as *C. "laevis"* and *Cladonia* sp. 1. In addition, some samples of *C. pocillum* and *C. pyxidata* were included, because these taxa could be phylogenetically close to the *C. humilis* complex (Stenroos et al. 2002). Samples of *C. hammeri* from both California and Mediterranean Europe were included in the analyses. Fresh material of *C. pulvinella*, suitable for molecular analyses, from California (where its type derives from) was not obtained. As outgroups, samples of *C. subturgida* Samp., *C. rangiformis* Hoffm., and *C. thomsonii* Ahti were chosen.

The species identifications in the *C. humilis* complex were mainly based on the podetium configuration, soredium size, presence of cortex on the podetia, and secondary metabolites, according to Hammer (1991), Ahti et al. (1995), Ahti (2000), and Ahti & Hammer (2002).

DNA extraction and amplification

Only one podetium of each specimen was selected for the DNA isolation. Before the DNA isolation, the secondary metabolites were extracted by soaking the samples in acetone for two hours, and the liquid was used for thin-layer chromatography (TLC). The DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) was used to extract DNA, according to the manufacturer's instructions. The DNA was dissolved in 200 l of buffer included in the kit. The four following loci were amplified: nuclear ITS rDNA using the primer pair ITS1F (Gardes and Bruns, 1993)/ITS4 (White et al. 1990) or SSU1780/LSU012 (Piercey-Normore & DePriest 2001), *rpb2* using the primer pairs RPB2-5F/RPB2-7R (Liu et al. 1999), RPB2dRaq/RPB2rRaq (Pino-Bodas et al. 2010a) or CLRPB2-5F/CLRPB2-7R (Yahr et al. 2006), *ef1α* using CLEF-3F/CLEF-3R (Yahr et al. 2006), and IGS rDNA using IGSf/IGSr (Wirtz et al. 2008). The amplifications of ITS rDNA, *rpb2* and *ef1α* were carried out with Ready-to-Go-PCR Beads (GE Healthcare Life Sciences, UK). The volume of each reaction was 25 l, with 0.4 mM final concentration of primers. The amplification of IGS rDNA was carried out using Biotaq polymerase (ECOGEN, Barcelona, Spain). The volume of reaction was 25 l, with 0.3 l of Taq polymerase, 2.5 l of 10X PCR buffer, 1.4 l of MgCl2 50 mM, 1.6 l of dNTPs (2.5 mM), 1 l of BSA (1mM), 1 l of each primer (10 mM) and 1 l of extracted DNA. The amplification programs were: 1) 94ºC for 5 min; 5 cycles of 94ºC for 30 s, 54ºC for 30 s and 72 ºC for 1 min; and 33 cycles of 94ºC for 30 s, 48ºC for 30 s and 72ºC for 1 min; with a final extension of 72ºC for 10 min for nuclear ITS rDNA, 2) initial denaturation at 94ºC for 5 min; 40 cycles of 95ºC for 1 min, 52ºC for 30 s and 72ºC for 2 min; with a final extension at 72ºC for 10 min for *rpb2* region, 3) initial denaturation at 94ºC for 5 min; 35 cycles of 95ºC for 1 min, 55ºC for 30 s and 72ºC for 1 min; with a final extension at 72ºC for 10 min for *ef1α* region, 4) 95ºC for 2 min; 35 cycles of 95ºC for 30 s, 54ºC for 30 s and 72 ºC for 1 min; with a final extension of 72ºC for 10 min. PCR products were purified using the QIAquick gel extraction Kit (QIAGEN, Hilden, Germany) or with ExoSAP-IT (USB Corporation, OH, USA). The sequencing was performed at Macrogen (South Korea) service (www.macrogen.com), with the same primers used for the PCR.

Phylogenetic analysis

The alignments were made manually with SE-AL v2.0a11 (Rambaut 2002) for each locus separately. Each region was analyzed by maximum parsimony (MP) and maximum likehood (ML). MP analyses were made using PAUP version 4.0.b.10 (Swofford 2002), using heuristic searches with 1000 random taxon-addition replicates with TBR branch swapping and MulTrees option in effect, equally weighted characters and gaps treated as missing data. For the confidence analysis, bootstrap was applied, with 1000 replicates, using the heuristic option. The ML analyses were implemented using RAxML 7.04 (Stamatakis 2006), assuming a GTRGAMMA model. Congruence among the different topologies inferred from the loci was tested following Lutzoni et al. (2004): each clade with more than 75% bootstrap support was scanned for conflict among loci. We considered the existence of a conflict whenever a clade was supported with a bootstrap (more than 75%) in a

Table 1. Samples included in this study with the GenBank accesion numbers.

Sample	Locality	Collector	Voucher specimen	ITS rDNA	IGS rDNA	*rpb2*	*ef1α*	clade
C. conista 1	Russia, Kursk Region, BuBel	*N. T. Zolotuchin*	H	JF926633	+	JF926568	JF926590	D
C. conista 2	USA, Connecticut, Litchfield county	*J. C. Lendemer et al.*	H	JF926634	+	JF926569	JF926591	D
C. conista 3	USA, New Jersey, Gloucester county	*J. C. Lendemer et al.*	H	JF926635	+	JF926570	JF926592	D
C. conista 4	USA, Pensylvania, Blair county	*J. C. Lendemer et al.*	H	JF926636	+	JF926571	JF926593	D
C. conista 5	Spain, Castellón, Chóvar,	*A. R. Burgaz*	MACB 97591	JF926612	+	JF926566	JF926588	D
C. conista 6	Spain, Huesca, Oza	*A. R. Burgaz*	MACB 92796	JF926613	+	JF926567	JF926589	D
C. conista 7	Spain, Orense, Cabeza de Manzaneda	*A. R. Burgaz*	MACB 98080	JF926630	+	—	JF926596	D
C. conista 8	Finland, Laatokan Karjala, Parikkala	*T. Ahti*	H	JF926629	+	JF926572	—	D
C. conista 9	Finland, Etelä-Häme, Nastola	*V. Haikonen*	H	JF926632	+	—	JF926594	D
C. conista 10	Russia, Sakha Republic, Yakutia	*T. Ahti*	H	JF926619	+	JF926573	JF926595	D
C. cyathomorpha 1	Spain, Ávila, Hoyocasero	*A. R. Burgaz*	MACB 97180	+	+	+	+	F
C. cyathomorpha 2	Spain, Madrid, Lozoya	*A. R. Burgaz*	MACB 97543	+	+	—	+	F
C. cyathomorpha 3	Norway, Hordaland, Bergen	*T. Ahti* 68660 &. *T. Tonsberg*	H	+	+	+	+	F
C. cyathomorpha 4	Portugal, Beira Alta, Penhas Douradas	*A. R. Burgaz*	MACB 101278	+	+	+	+	F
C. cyathomorpha 5	Spain, León, Igüeña	*A. R. Burgaz*	MACB 101279	+	+	+	—	F
C. cyathomorpha 6	Belgicum, Vielsalm, Cahay	*A. Aptroot* 67026	F	+	+	—	+	F
C. hammeri 1	USA, California, Sonoma county	*T. Ahti* 69200c	H	+	+	+	+	C
C. hammeri 2	USA, California, Sonoma county	*T. Ahti* 69200	H	+	+	+	+	C
C. hammeri 3	USA, California, Marin county	*T. Ahti* 69191	H	+	+	+	+	C
C. hammeri 4	USA, California, Russian river	*T. Ahti* 69198	H	+	+	+	+	C
C. hammeri 5	USA, California, Marin county	*T. Ahti* 68885	H	+	+	+	+	C
C. hammeri 6	USA, California, Marin county	*T. Ahti*	H	+	+	+	+	C
C. hammeri 7	USA, California, Sonoma county	*T. Ahti* 690201	H	+	+	+	+	C
C. hammeri 8	USA, California, Sonoma county	*T. Ahti* 690200	H	+	+	+	+	C
C. hammeri 9	USA, California, Peninsula Range	*K. Knudsen*	H		+	+	+	C
C. hammeri 10	USA, California,		H	+	+	+	+	C
C. hammeri 11	USA, California, River side county	*K. Knudsen* 6441.1	URC	+	+	+	+	C
C. hammeri 12	USA, California, River side county	*K. Knudsen* 11005	URC	+	+	+	+	C

Sample	Locality	Collector	Voucher specimen	ITS rDNA	IGS rDNA	*rpb2*	*ef1α*	clade
C. hammeri 13	USA, California, River side county	*K. Knudsen* 6441.4	URC	+	—	+	+	C
C. hammeri 14	USA, California, River side county	*J. C. Lendemer*	H	+	+	+	+	C
C. hammeri 15	USA, California, River side county	*J. C. Lendemer*	F	+	+	+	+	C
C. hammeri 16	Spain, Barcelona, Montseny	*A. R. Burgaz*	MACB 95732	+	+	+	+	B
C. hammeri 17	Portugal, Algarve, Monchique	*A. R. Burgaz*	MACB 97323	+	+	+	+	B
C. hammeri 18	Spain, Ávila, San Bartolomé de Bejar	*A. R. Burgaz*	MACB 96093	+	+	+	+	A
C. hammeri 19	Turkey, Giresum, Degirmenagzi	*K. Kinalioglu*	H	+	+	+	+	B
C. hammeri 20	Spain, Canary Island, Tenerife	*A. R. Burgaz*	MACB 102877	+	+	+	+	A
C. hammeri 21	Spain, Madrid, Somosierra	*A. R. Burgaz*	MACB 102876	+	+	+	+	B
C. hammeri 22	Andorra, Soldeu, Port d'Envalira	*A. R. Burgaz*	MACB 102875	+	+	+	+	-
C. hammeri 23	Bosnia and Herzegovina, Herzegovina-Neretva, Caplijina	*A. R. Burgaz*	MACB	+	+	+	+	B
C. humilis 1	Portugal, Trás-os-Montes, Rebordainhos	*A. R. Burgaz*	MACB 92885	JF926625	+	JF926584	JF926597	A
C. humilis 2	Spain, Toledo, Urda	*A. R. Burgaz*	MACB 92807	JF926622	+	JF926585	JF926598	A
C. humilis 3	Portugal, Baixo Alentejo, Cavalheiro	*A. R. Burgaz*	MACB 97326	JF926626	+	JF926587	JF926599	A
C. humilis 4	Portugal, Estremadura, Azoia	*A. R. Burgaz*	MACB 92818	JF926627	+	JF926583	JF926600	A
C. humilis 5	Spain, Madrid, La Acebeda	*A. R. Burgaz*	MACB 95913	JF926614	+	JF926575	JF926601	A
C. humilis 6	Spain, Gerona, Port de la Selva	*A. R. Burgaz*	MACB 95931	JF926615	+	JF926576	JF926602	A
C. humilis 7	Spain, Mallorca, Sa Pobla	*A. R. Burgaz*	MACB 92803	JF926628	+	JF926581	JF926603	A
C. humilis 8	Portugal, Madeira, Funchal	*P. Alanko*	H	JF926616	+	JF926582	JF926604	A
C. humilis 9	Turkey, Trabzon, Akcaabat	*K. Yazici*	H	JF926617	+	JF926577	JF926605	A
C. humilis 10	USA, California, Los Angeles	*K. Knudsen*	H	JF926618	+	JF926578	JF926606	A
C. humilis 11	USA, California, Sonoma County	*T. Ahti*	H	JF926620	+	JF926579	JF926607	A
C. humilis 12	Croatia, Dubrovnik-Neretva, Zamaslina	*A. R. Burgaz*	MACB 101103	JF926621	+	JF926580	JF926608	A
C. humilis 13	USA, California, W of Rock Spring	*T. Ahti* 68970 & *L. St. Clair*	H	JF926624	+	JF926574	JF926610	A
C. humilis 14	USA, California, Marin County	*T. Ahti* 68963 & *L. St. Clair*	H	JF926623	+	JF926586	JF926609	A
C. humilis 15	Taiwan, Nantou County	*A. Aptroot*	H	+	—	+	+	A
C. humilis 16	Spain, Gerona, Olot	*A. R. Burgaz*	MACB	+	+	—	+	A
C. kurokawae 1	Japan, Kyushu, Bungo	*H. Kashwadani, Y. Umezu & K. Umezu*	H	+	+	—	—	E
C. kurokawae 2	Japan, Honshū, Ibaraki Pref	*T. Ahti*	H	+	+	+	+	E

Sample	Locality	Collector	Voucher specimen	ITS rDNA	IGS rDNA	*rpb2*	*ef1α*	clade
C. kurokawae 3	China, Yunnan, Heqin County	*A. Aproot*	H	+	+	+	+	E
C. kurokawae 4	China, Hunan, Sang-Zhi County	*Koponen et. al.* 55724	H	+	+	+	+	E
C. "laevis" 1	Spain. Burgos, Urrez	*A. R. Burgaz*	MACB	+	+	+	+	A
C. "laevis" 2	Spain, Albacete, Villapalacios	*A. R. Burgaz*	MACB 102944	+	+	+	+	A
C. "laevis" 3	Spain, Murcia, Alhama de Murcia	*A. R. Burgaz*	MACB 102945	+	+	+	+	A
C. "laevis" 4	Portugal, Alto Alentejo, Bencatel	*A. R. Burgaz*	MACB 102946	+	+	+	+	A
C. "laevis" 5	Portugal, Algarve,	*A. R. Burgaz*	MACB 102882	+	+	+	+	A
C. nashii 1	USA, California, Santa Rosa Island (Topotype)	*K. Knudsen*	H	+	+	+	—	G
C. nashii 2	USA, California, Marin County	*T. Ahti* 68967 & *L. St. Clair*	H	+	+	+	+	G
C. nashii 3	USA, California, Sonoma County	*T. Ahti* 69200a	H	+	+	+	+	G
C. nashii 4	Mexico, Baja California, San Quintín peninsula	*T. Ahti*	H	+	+	+	—	G
C. pocillum 1	USA, Minnesota, Wabasha County		S L3680	+	+	+	+	H
C. pocillum 2	USA, Connecticut, Litchfield county	*J. C. Lendemer* & *A. L. A. Foray*	UPS L-160151	+	+	+	+	H
C. pocillum 3	France, Corsica, Cinto	*E. Granda*	MACB 102880	+	+	+	+	H
C. pulvinella 1	Spain, Sevilla, Alanis	*A. R. Burgaz*	MACB 93015	+	+	+	+	A
C. pulvinella 2	Spain, Menorca, Es Mercada	*A. R. Burgaz*	MACB 98059	+	+	+	+	A
C. pulvinella 3	Portugal, Estremadura, Serra da Sintra	*A. R. Burgaz*	MACB 92820	+	+	—	+	A
C. pulvinella 4	Spain, Almería, Rodalquilar	*A. R. Burgaz*	MACB 97949	+	+	+	+	A
C. pyxidata 1	Ukranie, Luhans`k Oblast, Pereval`s`k district	*O. Nadeina*	H	+	+	+	+	H
C. pyxidata 2	Greenland, Qeqertaq	*E. S. Hansen*	H	+	+	+	+	H
C. subconistea 1	North Korea, Gyonggy, Uhwang	*K. H. Moon*	H	+	+	+	+	E
C. subconistea 2	China, Hunan, Yan-Ling Co.	*Koponen et al.* 55878	H	+	+	+	+	E
Cladonia sp. 1	Spain, Granada, Loja	*A. R. Burgaz*	MACB 94396	+	+	+	+	A
Cladonia sp. 2	Spain, Tarragona, Corberá d'Ebre	*A. R. Burgaz*	MACB 93167	+	+	+	+	A

Sample	Locality	Collector	Voucher specimen	ITS rDNA	IGS rDNA	*rpb2*	*ef1α*	clade
Cladonia sp. 3	Spain, Mallorca, Buyola	*A. R. Burgaz*	MACB 102879	+	+	+	+	B
Cladonia sp. 4	Croatia, Dubrovnik-Neretva, Palje Brdo	*A. R. Burgaz*	MACB 101113	+	+	+	+	B
Cladonia sp. 5	Bosnia and Herzegovina, Srpska Republic, Trebinje	*A. R. Burgaz*	MACB 102885	+	+	+	+	B
Cladonia sp. 6	Cabo Verde, St. Antao, Porto Novo	M. P. Martín *3262*	MA-lichen	+	+	+	+	A
Cladonia sp. 7	Cabo Verde, St. Antao, Porto Novo	*M. P. Martín* 3263	MA-lichen	+	+	+	+	A
C. rangiformis	Spain, Menorca, Ferreires	*A. R. Burgaz*	MACB 96193	JF288804	JN811366	JF288839	JN811444	-
C. subturgida	Spain, Ciudad Real, Villamanrique	*A. R. Burgaz*	MACB 99488	JF288793	+	JF288824	+	-
C. thomsonii	Russia, Krasnoyarsk, Severmaya Zemlya archipelago	*M. Zhurbenko*	H	JN811402	JN811369	JN811431	+	-

locus, but it was not supported in other locus and the individual sequences of this clade are part of another clade with bootstrap support ≥ 75 %.

The combined dataset was analyzed by MP, ML and Bayesian approach. The combined dataset was partitioned in 8 partitions for ML and Bayesian analyses: ITS rDNA, IGS rDNA and each of three codon positions of *rpb2*, and each of three codon positions of *ef1α*, respectively. Bayesian analysis was carried out using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). The models applied to each partition were selected by MrModeltest (Nylander, 2004) under AIC criterion, and are listed in Table 2. The posterior probabilities were approximated by sampling trees using Markov Chain Monte Carlo (MCMC). The posterior probabilities of each branch were calculated by counting the frequency of trees visited during MCMC analysis. Two simultaneous runs with 20,000,000 generations, each starting with a random tree and employing 4 simultaneous chains, were executed. Every 1000th tree was saved into a file. The first 2,000,000 generations (i.e., the first 2000 trees) were deleted as the "burn in" of the chain. AWTY (Nylander et al. 2008) was used to determine when the chains reached the stationary stage. The 50% majority-rule consensus tree was calculated using the "sumt" command of MrBayes.

The following hypotheses were tested: 1) monophyly of *C. humilis*, 2) monophyly of *C. pulvinella*, 3) monophyly of *C. kurokawae*, 4) monophyly of *C. subconistea*, 5) monophyly of *C. hammeri*, 6) monophyly of *C. hammeri* samples from Europe, 7) monophyly of *C. "laevis"*, and 8) monophyly of *Cladonia* sp. Alternative hypothesis tests included the Shimodaira-Hasegawa test (SH; Shimodaira and Hasegawa 1999) and expected likelihood weight (ELW; Strimmer & Rambaut 2002) that were performed using TREE-PUZZLE 5.2 (Schmidt et al. 2002).

Table 2. Phylogenetic information of each locus and evolutionary models chosen by MrModeltest.

Datasets	Sites	Informatives sites	Nº of equally MP trees	Tree length	LnL	Model
ITS rDNA	658	102	1000	272	-2638.096	SYM+G
IGS rDNA	335	87	1000	182	-1448.375	GTR+G
rpb2	870	158	1000	290	-2940.927	SYM+G
ef1a	611	77	8	135	-1676.098	HKY+I

Genealogical sorting index

Whenever the morphological species were not monophyletic, the genealogical sorting index (GSI) was used to assess the level of genealogical exclusivity for each putative species (Cummings et al. 2008). The GSI was calculated for ML tree of each locus. Each branch tip was assigned a group name, corresponding to morphological species (*C. humilis, C. pulvinella, C. subconistea, C. kurokawae, C. hammeri*, *C.* "*laevis*"). This method was also applied to test the monophyly of *C. hammeri* samples from Europe. The GSI was calculated using 10.000 permutations on the online platform: www.genealogicalsortingindex.org.

Genetic distances and fixation index (F_{ST})

The pairwise distances using the HKY + G + I model were calculated in PAUP*. According to Del-Prado et al. (2010) the intraspecific distances were calculated as the mean value of the pairwise distances between the samples of each species. The interclade distances were calculated as the mean value of the pairwise distances between the samples of two different clades.

The pairwise fixation index F_{ST} (Weir & Cockerham 1984) was calculated with DnaSPv. 5 (Librado & Rozas 2009) for each locus. According to Spribille et al. (2011) that used the F_{ST} value to estimate the genetic differentiation between clades.

Morphology and chemistry study

The specimens were studied under a dissecting scope; the morphology of the podetia and their plates, soredia and granules were observed. Measurements of podetium length and scyphus width were taken. Microscopic measurements of the soredia, podetial wall thickness and each anatomical layer of the podetia were taken in every sample at x400 by a light microscope. The hand-cut sections were made at the podetial base and mounted in distilled water. The secondary metabolites were checked by TLC according to the standardized procedures of White & James (1985), with solvent systems A and B. The fatty acids were visualized before developing the plates by heating.

Results

Phylogenetic results

In this study 278 new sequences were generated. Sequence features for the individual genes, parsimony information and the models of sequence evolution for each partition are listed in Table 2. The locus with most informative positions percentage was IGS rDNA (25.97%), followed by *rpb2* (18.16%) ands ITS rDNA (15.50%); *ef1α* was the less informative (12.60%). The topology of MP trees and ML trees for each locus was similar. The results of ITS rDNA and *rpb2* showed 8 clades corresponding to: 1) *C. nashii*, 2) samples of *C. hammeri* from North America, 3) *C. cyathomorpha*, 4) *C. conista*, 5) samples of *C. subconistea* and *C. kurokawae*, 6) samples of *C. humilis*, *C. pulvinella*, *C. hammeri* from Europe, *C. "laevis"* and *Cladonia sp.* 7) samples of *C. hammeri* from Europe and *Cladonia sp.* 8) samples of *C. pyxidata* and *C. pocillum*. In the trees of IGS rDNA, *C. conista* did not form a monophyletic group, and in *ef1α* trees all the samples of *C. hammeri* from Europe and *Cladonia sp.* appeared in the same clade of *C. humilis*, *C. pulvinella* and *C. "laevis"*.

No conflict among the loci was found and the four genes were combined. The combined dataset contained 2474 characters, 1857 of which were constant, and 424 parsimony-informative. MP analysis generated 1000 equally parsimonious trees, 942 steps long, with a value CI = 0.7304 and RI = 0.9391. ML analysis yielded a tree with a likelihood value of LnL = -9452.597, while the mean likelihood of the Bayesian tree sampling was LnL = -9558.74. The trees of the three analyses had similar topology. *C. nashii* did not appear closely related to the other species of the *Cladonia humilis* complex (Fig. 1). Eight monophyletic well supported clades appeared, the clade A constituted by samples of *C. humilis*, *C. pulvinella*, some samples of *C. hammeri* from Europe, *C. "laevis"* and some samples of *Cladonia* sp.; the clade B formed by samples of *C. hammeri* from Europe and samples of *Cladonia* sp. and closely related with clade A*;* the clade C formed by samples of *C. hammeri* from North America; the clade D that corresponded to *C. conista,* closely related to clade C*;* the clade E included the samples of *C. kurokawae* and *C. subconistea*; the clade F corresponded to *C. cyathomorpha.* This clade appeared at the base of *C. humilis* complex; the clade G constituted by *C. nashii*; the last clade (clade H) was formed by *C. pyxidata* and *C. pocillum* samples. One sample of *C. hammeri* from Andorra appeared closely related to the clade of *C. kurokawae* and *C. subconistea.*

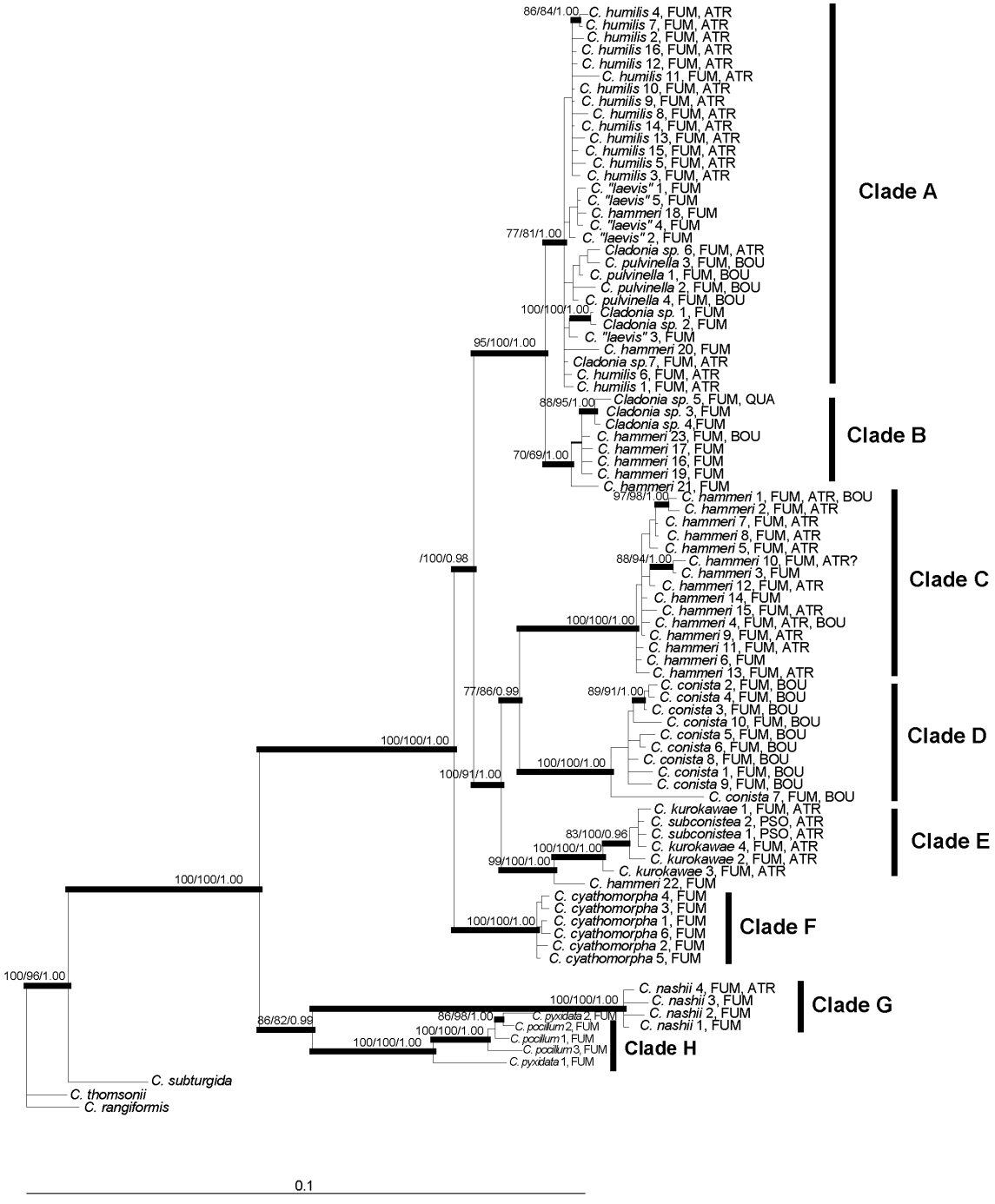

Fig 1. Phylogeny of the *Cladonia humilis* complex based on a combined dataset (ITS rDNA, IGS rDNA, *rpb2* and *ef1α*). This is a 50% majority rule consensus tree of a Bayesian analysis. Branches supported with posterior probability ≥ 0.95 and bootstrap ≥ 70 % are indicated in bold. ATR = atranorin, BOU = bourgeanic acid, FUM = fumarprotocetraric acid, PSO = psoromic acid, QUA = quasigenic acid.

Hypothesis contrast and GSI

The SH and ELW tests rejected the monophyly of all the samples of *C. hammeri*, as well the monophyly of the samples of *C. hammeri* coming from Europe; the monophyly of *Cladonia* sp. (Table 3) was also rejected by both tests. The monophyly of *C.*

subconistea was rejected by the SH test, but not by the ELW test. On the contrary, the monophyly of *C. pulvinella* was rejected by the ELW test, but not by the SH test. As regards *C. humilis, C. kurokawae* and *C. "laevis"* none of the tests rejected the monophyly of these morphospecies.

The support of the monophyly by the GSI test varied according to the distinct genes (Table 4). Only for two species, *C. humilis* and *C. hammeri,* the GSI P-value was significant in all the four genes, but with some moderate values (<0.7). ITS rDNA and IGS rDNA showed genealogical divergence of *C. kurokawae*, but *rpb2* and *ef1α* did not. Only in ITS rDNA the GSI P-value indicated that *C. pulvinella* and the samples of *C. hammeri* coming from Europe were close to reach the monophyly. The GSI P-value rejected the monophyly of *C. subconistea*, *C. "laevis"* and *Cladonia* sp for all the loci.

3.3. Genetic divergence and fixation index

The genetic distances within the clades showed a variation of 0.0016-0.0101 in ITS rDNA, 0.0031-0.0123 in IGS rDNA, 0.0016-0.0296 in *rpb2*, and 0.0-0.012 in *ef1α* (Table 5). The greatest distances within the clades occurred between clade A sequences for ITS rDNA and *rpb2* regions, between clade B sequences for IGS rDNA and between clade G sequences in *ef1α*. The genetic distances inter-clades varied 0.0209-0.1042 in ITS rDNA, 0.0162-0.4439 in IGS rDNA, 0.0194-0.1103 in *rpb2* and 0.0013-0.0781 in *ef1α*. The greatest divergence among lineages was found between A and E clades in IGS rDNA (0.4439).

F_{ST} values are displayed in table 6. In most of comparisons between loci the values exceeded 0.5, except between clades A and B, where the values were 0.06183 in *rpb2* and 0.0 en *ef1α*. F_{ST} value between A and B clades in ITS rDNA and IGS rDNA was, however, 0.90441 and 0.68091 respectively.

Table 3. Results of alternatives topologies test, Shimodaira-Hasegawa test (SH) and likelihood weight test (ELW). * $P \leq 0.05$, statistically significant results.

Hypotheses	LnL	SH	ELW
C. hammeri is monophyletic	-10112.72	0.0000*	0.0000*
C. humilis is monophyletic	-9803.52	0.5900	0.2279
C. kurokawae is monophyletic	-9833.38	0.4490	0.0686
C."laevis" is monophyletic	-9821.89	0.0780	0.0697
C. pulvinella is monophyletic	-9817.73	0.6790	0.0167*
Cladonia sp. is monophyletic	-9968.38	0.0000*	0.0000*
C. subconistea is monophyletic	-9802.56	0.9700*	0.4114
C. hammeri from Europe is monophyletic	-9921.27	0.0010*	0.0000*

Morphological and chemical variation of the clades

Table 7 outlines the morphological variation found within each clade. Clade A contains samples with farinose soredia (samples identified as *C. humilis*), samples with granular soredia (identified as *C. hammeri* and *C. pulvinella*), samples with micro-squamules (labelled as *Cladonia sp.*) and even samples that contain farinose soredia in the external side of the podetium and granules inside the scyphi (labelled as *C. "laevis"*). As for the cortex morphology, clade A contains samples with a smooth cortex, others with areolate cortex and others that lack cortex or, if present, is limited to podetial base (*C. pulvinella*). Regarding chemical variability, samples are found with fumarprotocetraric acid and atranorin; with fumarprotocetraric and bourgeanic acids; and with fumarprotocetraric acid only (Fig. 1). Clade B contains samples with granules (*C. hammeri*) and samples with micro-plates (*Cladonia sp.*); samples with smooth cortex reaching near the podetial tip and samples with areolate cortex similar to that of *C.*

pyxidata and *C. pocillum*. The samples within this clade only contain fumarprotocetraric acid. Clade C is morphologically more homogeneous that the previous ones; its samples show granules, with areolate cortex in the base of the podetia (Fig. 2). Nevertheless, this clade is heterogeneous as to secondary metabolites, with samples that only contain fumarprotocetraric acid, others with fumarprotocetraric acid and atranorin, and others with fumarprotocetraric acid, atranorin and bourgeanic acid.

Table 4. Genealogical sorting index and probability values under hypothesis null that the samples labed as putative species are monophyletic. In bold the significant results.* $P \leq 0.001$.

Putative species	ITS rDNA		IGS rDNA		*rpb2*		*ef1a*	
	GSI	P	GSI	P	GSI	P	GSI	P
C. hammeri	**0.597**	**9.9e-05***	**0.373**	**9.9e-05***	**0.374**	**9.9e-05***	**0.423**	**9.9e-05***
C. humilis	**0.577**	**9.9 e-05***	**0.4**	**2.9 e-04***	**0.505**	**9.9e-05***	**0.343**	**7.9 e-04***
C. kurokawae	**0.732**	**4.9 e-04***	**0.571**	**6.9 e-04***	0.3	0.026	0.651	0.002
C. "laevis"	0.286	0.015	0.175	0.124	0.16	0.186	0.119	0.289
C. pulvinella	**0.732**	**2.9 e-04***	0.158	0.181	0.183	0.141	0.083	0.568
***Cladonia* sp.**	0.276	0.012	0.278	0.010	0.333	0.004	0.08	0.770
C. subconistea	0.318	0.061	0.233	0.109	0.488	0.034	1	0.010
***C. hammeri* only samples**	**0.456**	**9.9 e-04***	0.176	0.161	0.111	0.746	0.233	0.026

Clade D includes samples with farinose soredia, smooth cortex, and containing only fumarprotocetraric and bourgeanic acids. Samples in clade E have farinose to granular soredia and a smooth or areolate cortex. As for secundary metabolites, samples are found which contain fumarprotocetraric acid and atranorin, while others have atranorin and psoromic acid. Clade F contains samples with granules and areolate cortex. All the samples in this clade contain fumarprotocetraric acid. Clade G includes samples with farinose soredia that cover most of the podetial surface, sometimes with a corticate inferior half (Fig. 2). This clade comprises samples that only contain fumarprotocetraric acid and samples with both fumarprotocetraric acid and atranorin.

Table 5. Genetic distances amoung and within the the clade for every locus. Mean ± standar desviation.

Group	ITS rDNA	IGS rDNA	*rpb2*	*ef1α*
Clade A	0.0101 ± 0.0142	0.0067 ± 0.0077	0.0296 ± 0.0767	0.0015 ± 0.0022
Clade B	0.0025 ± 0.0014	0.0123 ± 0.0160	0.0025 ± 0.0014	0.0012 ± 0.0015
Clade C	0.0034 ± 0.0043	0.0110 ± 0.0087	0.0034 ± 0.0043	0.0
Clade D	0.0033 ± 0.0022	0.0073 ± 0.0088	0.0107 ± 0.0151	0.0077 ± 0.0063
Clade E	0.0036 ± 0.0016	0.0031 ± 0.000004	0.0055 ± 0.0057	0.0056 ± 0.0045
Clade F	0.0016 ± 0.0011	0.0063 ± 0.0029	0.0016 ± 0.0011	0.0
Clade G	0.0079 ± 0.0041	0.0047 ± 0.00001	0.0079 ± 0.0417	0.012
Clade A- Clade B	0.0292 ± 0.0074	0.0304 ± 0.0098	0.0375 ± 0.0521	0.0013 ± 0.0023
Clade A- Clade C	0.0291 ± 0.0078	0.0618 ± 0.0093	0.0374 ± 0.0521	0.0140 ± 0.0010
Clade A- Clade D	0.0301 ± 0.0046	0.0280 ± 0.0065	0.0421 ± 0.0521	0.0207 ± 0.0050
Clade A- Clade E	0.0303 ± 0.0059	0.4439 ± 0.0328	0.0401 ± 0.0553	0.0212 ± 0.0052
Clade A- Clade F	0.0215 ± 0.0042	0.0362 ± 0.0042	0.0298 ± 0.0493	0.0091 ± 0.0014
Clade A- Clade G	0.1042 ± 0.0214	0.2018 ± 0.0050	0.1103 ± 0.0436	0.0645 ± 0.0017
Clade D- Clade B	0.0330 ± 0.0025	0.0203 ± 0.0090	0.0361 ± 0.0096	0.0210 ± 0.0030
Clade D- Clade C	0.0314 ± 0.0042	0.0397 ± 0.0112	0.0344 ± 0.0103	0.0100 ± 0.0039
Clade D- Clade E	0.0178 ± 0.0024	0.0240 ± 0.0052	0.0222 ± 0.0125	0.0182 ± 0.0061
Clade D- Clade F	0.0250 ± 0.0028	0.0162 ± 0.0091	0.0281 ± 0.0104	0.0261 ± 0.0038
Clade D- Clade G	0.0953 ± 0.0172	0.1831 ± 0.0077	0.0991 ± 0.0216	0.0720 ± 0.00008
Clade F- Clade B	0.0233 ± 0.0115	0.0268 ± 0.0060	0.0194 ± 0.0015	0.0090 ± 0.0012
Clade F- Clade C	0.0252 ± 0.0041	0.0455 ± 0.00007	0.0252 ± 0.0041	0.0183 ± 0.0012
Clade F- Clade E	0.0209 ± 0.0016	0.0318 ± 0.0030	0.0216 ± 0.0032	0.0268 ± 0.0043
Clade F- Clade G	0.0942 ± 0.0222	0.1902 ± 0.0037	0.0942 ± 0.0222	0.0650 ± 0.0155
Clade C- Clade B	0.0311 ± 0.0037	0.0532 ± 0.0109	0.0311 ± 0.0037	0.0138 ± 0.0017
Clade C- Clade E	0.0289 ± 0.0038	0.0511 ± 0.00009	0.0289 ± 0.0038	0.0112 ± 0.0041
Clade C- Clade G	0.0974 ± 0.0221	0.2269 ± 0.0080	0.0984 ± 0.0221	0.0660 ± 0.0037
Clade B- Clade E	0.0330 ± 0.0019	0.0315 ± 0.0079	0.0327 ± 0.0022	0.0220 ± 0.00001
Clade G- Clade B	0.0939 ± 0.0184	0.1913 ± 0.0064	0.0939 ± 0.0184	0.0642 ± 0.0014
Clade G- Clade E	0.0935 ± 0.0149	0.2072 ± 0.00001	0.0935 ± 0.0149	0.0781 ± 0.00003

Table 6. Pairwise Fst for each dataset (ITS rDNA, IGS rDNA, *rpb2* and *ef1α*). In bold the low values.

Comparations	ITS rDNA	IGS rDNA	*rpb2*	*ef1α*
Clade A-Clade B	0.90441	0.68091	**0.06183**	**0.000**
Clade A-Clade C	0.90653	0.85448	0.90687	0.74802
Clade A-Clade D	0.70509	0.80749	0.94996	0.77683
Clade A-Clade E	0.88613	0.88526	0.92886	0.84157
Clade A-Clade F	0.89314	0.81822	0.97362	0.92070
Clade A-Clade G	0.96130	0.96569	0.98679	0.85202
Clade B-Clade C	0.91959	0.77386	0.89058	0.74025
Clade B-Clade D	0.74742	0.56166	0.93326	0.77319
Clade B-Clade E	0.90689	0.75045	0.90991	0.83682
Clade B-Clade F	0.91016	0.64429	0.95709	0.91429
Clade B-Clade G	0.95591	0.94513	0.97511	0.85065
Clade C-Clade D	0.74461	0.80254	0.88760	0.52016
Clade C-Clade E	0.90948	0.85836	0.87636	0.51168
Clade C-Clade F	0.91256	0.80551	0.91316	0.89619
Clade C-Clade G	0.96151	0.95704	0.94578	0.83382
Clade D-Clade E	0.51969	0.84058	0.92308	0.62055
Clade D-Clade F	0.70968	0.59322	0.96350	0.85146
Clade D-Clade G	0.90230	0.97170	0.97203	0.79840
Clade E-Clade F	0.83636	0.84746	0.93855	0.92308
Clade E-Clade G	0.96378	0.97638	0.96698	0.84729
Clade F-Clade G	0.96000	0.96441	0.99363	0.88095

Table 7. Morphological variation found in each clade of phylogenetic analyses.

Character	Clade A	Clade B	Clade C	Clade D	Clade E	Clade F	Clade G
Podetia morphology	Gradually flaring	Gradually flaring	Sharply flaring	Long stalk	Sharply flaring	Gradually flaring	Sharply flaring
Podetia size (mm)	2-9	2.8-8	3-10	4.75-12	2.9-8	4-12	3-5.5
Thickness (µm)	145-240	245-300	140-290	160-338	145-370	145-300	230-425
Scyphi width (mm)	1-7	1.2-7	2-5.5	3.25-7.25	1-4	1.5-4	2-4
Cortex	Smooth, areolated, extends higher, at the base	Smooth or verrucose, extends higher	Verrucose, limited at base or half-podetia	Smooth, extends higher	Smooth, areolated-corticate, extends higher	Areolated, verrucosed, half-podetia	Lack or only at the base
Soredia size	20-255 µm	40-175 µm	50-250 µm	20-45 µm	17-52 µm	45-390 µm	20-80 µm
Chemistry	I) FUM, PRO, ATR II) FUM, PRO, BOU III) FUM, PRO	I) FUM, PRO	I) FUM, PRO II) FUM, PRO, ATR III) FUM, PRO, ATR, BOU	I) FUM, PRO, BOU	I) FUM, PRO, ATR II) PSO, CPSO, ATR	I) FUM, PRO	I) FUM, PRO II) FUM, PRO, ATR
Distribution	North and central America, Europe and Asia	Mediterranean Europe	North America	Europe and North America	East Asia	Europe	North America

Discussion

Species delimitation in the *Cladonia humilis* complex

The *C. humilis* complex turned out to be not monophyletic; *C. nashii* does not group with the remaining species in the complex. We found that the *C. humilis* complex was solved into eight monophyletic well supported clades. But not all these clades correspond to previously described species nor are all morphologically or chemically homogeneous. Clade A contains the highest number of specimens and the largest morphological diversity. It comprises samples previously identified as *C. humilis*, *C.*

pulvinella, *C. hammeri*, *C. "laevis"*and *Cladonia sp.* Genetic distances within clade A for ITS rDNA and *rpb2* markers are similar to those found among the clades, which could indicate the presence of a species complex (Del-Prado et al. 2010). In phylogenetic analyses based on the four loci, *C. humilis* was not solved as a monophyletic entity. However, neither the SH test nor the EWL test reject the monophyly of *C. humilis,* and GSI significantly supported the monophyly of *C. humilis* in all the four loci. Morphologically the specimens of this taxon are easy to distinguish from the remaining samples in clade A by their smooth cortex and farinose soredia (Ahti 1966). The lack of monophyly is probably due to incomplete lineage sorting. In view of the results, we recommend to keep the species rank for *C. humilis*.

Cladonia pulvinella was not solved as a monophyletic entity; the ELW test rejected the monophyly of this morphospecies, and GSI only supported monophyly in ITS rDNA. Consequently, our results do not clearly support the acceptance of *C. pulvinella*. It is necessary, however, to emphasize that in our material *C. pulvinella* is only represented by European samples, while the verification of their similarity with the samples from California, where the species was originally described, is still pending. Therefore, although recognized from Europe by Burgaz & Ahti (2009), for instance, the identity of the European *C. pulvinella* is still uncertain.

The samples provisionally identified as *C. "laevis"* did not represent a monophyletic clade, and GSI did not support monophyly, but hypothesis tests did not reject the monophyly of these samples. Faced with contradictory results, we prefer not to acknowledge *C. "laevis"* as a new species, and we expect to collect new data. Anyway, we underline that these samples differ both morphologically and chemically from *C. humilis* s.str.

Cladonia hammeri turned out to be polyphyletic. The samples were divided into three clades. Clade C exclusively contains samples of *C. hammeri* coming from California, where this species was originally described (Ahti & Hammer 2002). Clades A and B contain samples of *C. hammeri* coming from Europe intermingled with specimens of other species. Hypothesis contrasts reject the monophyly of *C. hammeri* s.l., but GSI significantly supported the species monophyly in the four markers. F_{ST} values between clade C and clades A and B were high (Table 6), being the lowest found value 0.74025 (between clades B and C for *ef1α*). This result indicates the absence of genetic flow between clade B and the remaining clades containing samples of *C. hammeri* coming from Europe. The samples collected in Europe are morphologically different from the ones from North America. The European samples have a smooth cortex that almost reaches the scyphal rim, similarly to that of *C. humilis* and *C. conista* (Tønsberg 1979; Laundon 1984). The podetia of the North American samples are mostly covered by granules, while the cortex is limited to the base or the inferior half of the podetia, and it is not smooth, concordant with the original description of the species (Ahti & Hammer 2002). Based on our results, we consider the populations of *C. hammeri* from North America to constitute a species different from those of Europe. It is no wonder, because from the very beginning the identity of the European material with *C. hammeri* has been uncertain. The chemical diversity of clade C was really great (Fig. 1). The only secondary metabolite up to date found in *C. hammeri* was fumarprotocetraric acid (Ahti & Hammer 2002). However, here we have found that in addition to this substance atranorin, or atranorin along with bourgeanic acid also can be present.

The taxonomic identity of the European samples, previously identified as *C. hammeri*, is more difficult to interpret. These samples are not monophyletic. On the

other hand, the monophyly of *C. hammeri* samples coming from Europe was rejected by the SH and ELW tests, while GSI only supported the monophyly of these samples for

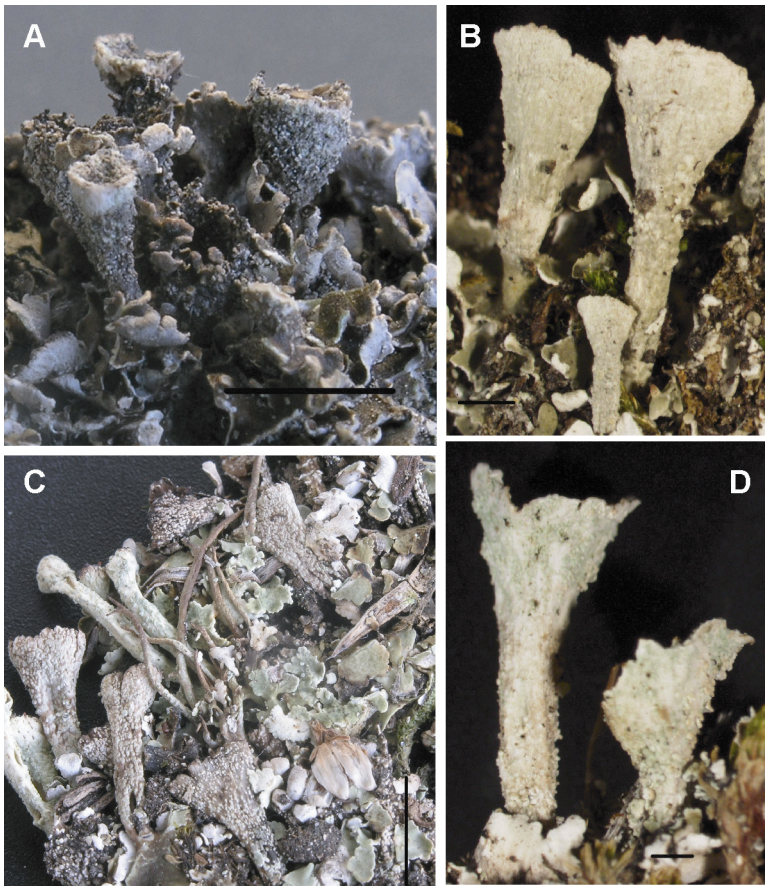

Fig. 2. Morphology of some species of *C. humilis* complex a) *C. nashii* (bar = 5 mm), b) *C. subconistea* (bar = 1 mm) c) *C. cyathomorpha* (Bar = 5 mm) d) *C. hammeri* (Bar = 1 mm).

ITS rDNA. F_{ST} values between clades A and B (which contains samples of *C. hammeri*) in ITS rDNA and IGS rDNA indicate genetic divergence, unlike *rpb2* and *ef1α* (Table 6), whose values are near 0, which seems to suggest the existence of gene flow between clades. These results do not clearly prove that *C. hammeri* from Europe is an independent species. What can surely be concluded is that the European samples of *C. hammeri* differ genetically from the North American ones. In some groups of Parmeliaceae (Divakar et al. 2007, 2010) a similar pattern has been found: morphologically similar populations in Europe and California that differ genetically.

The samples of *Cladonia* sp. did not form a monophyletic group. Hypothesis tests and GSI neither supported the monophyly of these samples. Morphologically it is impossible to distinguish the samples which laid in clade A from the ones which laid in clade B. All of them show a verruculose-areolate cortex that differentiate them from the samples of *C. hammeri* coming from Europe. Nevertheless, some of the samples grouped together with the European samples of *C. hammeri* in clade B. While only the mentioned data are available, we refrain from drawing any taxonomical conclusions.

The clade D, well supported in the three phylogenetic analyses of the combined matrix, and with high F_{ST} values for the three markers, corresponds to *C. conista*. This taxon is morphologically difficult to distinguish from *C. humilis* s. str., and they have often been treated as conspecific (Ahti 2000; James 2009). But in molecular analyses

Dolnik et al. (2010) and Pino-Bodas et al. (2012) found that these species are genetically different. Our results confirm this conclusion.

Primarily owing to the presence or absence of psoromic acid *C. subconistea* and *C. kurokawae* have been considered as separate species (Ahti et al. 1995; Awasthi & Ahti 2007). However, the clade E, strongly supported in all phylogenetic analyses, includes intermingled samples of both species. SH test rejected the monophyly of *C. subconistea,* and GSI was not statistically significant for any of the markers. Intra-clade genetic distances were similar to the ones found in other clades constituted by a unique species (Table 5), what leads us to suspect that this clade comprises one only species. In addition, *C. subconistea* and *C. kurokawae* are sympatric in China, Japan and Korea (Awasthi & Ahti 2007). Therefore, this study indicates that *C. kurokawae* and *C. subconistea* are conspecific, being two chemotypes of the same species. When united the correct name is *C. subconistea*.

Some doubts have been expressed whether *C. cyathomorpha* is a well-delimited unit (Burgaz & Ahti 2009; James 2009), owing to the fact that it is morphologically variable, especially with respect to the primary thallus. In this study we tried to include the whole morphological variation described for the species: samples with the lower side of squamules venose or sorediate or corticate (Fig. 3). All of them grouped together into one monophyletic clade (clade F). Therefore, the differences in primary thallus only represent intraspecific variation. Similar presence or absence of soredia on primary thallus squamules does occur in other species of *Cladonia*, as *C. acervata* S. Hammer (Hammer 2001), *C. borbonica* Nyl. (Ahti 2000), *C. coniocraea* (Flörke) Spreng. (Ahti & Hammer 2002), or *C. corniculata* Ahti & Kashiwadani (Ahti 2000); in some of them it is a developmental stage, while a corticate lower side is a character rare in *Cladonia* (Ahti 2000). In most cases this taxon is easily distinguished from the other species within the group by the presence of pale pink-coloured veins on the lower face of the primary thallus. The material studied here only contains fumarprotocetraric acid; the substance identified by Jølle (1977) was not found in the present study material (but has been noted in other specimens). Its constant presence is hardly a diagnostic character of the species.

Clade G contains all the samples identified as *C. nashii*; all of these present farinose soredia (Table 7), but not all contain fumarprotocetraric acid and atranorin, which was the originally described chemotype of this species (Ahti & Hammer 2002). The presence of atranorin seems to be inconstant. Morphologically this species is rather similar to the others in the *C. humilis* complex, but does not form a monophyletic group with them. The new *Cladonia* phylogeny (Stenroos et al. in prep.) will surely show which species are closely related to *C. nashii.*

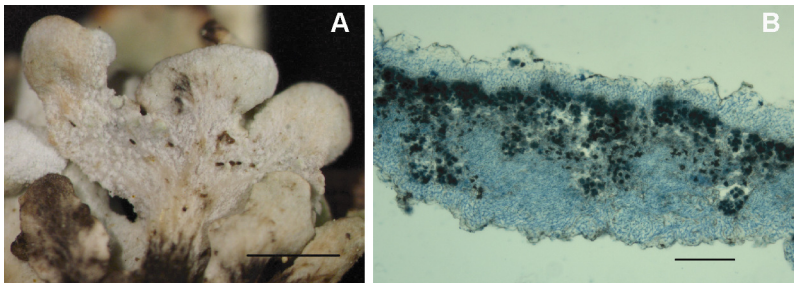

Fig. 3. Features of *C. cyathomorpha* a) Veins on lower face of the primary thallus (Bar = 1 mm) b) Cross-section of the primary thallus stained with laftophenol blue solution (Bar = 50 μm).

Clade H contains samples identified as *C. pyxidata* or *C. pocillum*. Our results agree with those by Kotelko & Piercey-Normore (2010), who on the basis of mainly Canadian material reported that neither species is monophyletic. The *C. pyxidata* group needs a through taxonomic revision based on larger material.

Significance of phenotypic characters in the *C. humilis* complex

The phylogenetic results show that the phenotypic features often do not characterize monophyletic lineages. As to single characters, soredium size has been widely used to distinguish between closely related species in *Cladonia* (Hennings 1983). Occasionally even a correlation between soredium size and secondary metabolites has been found (Ahti 1966; Kristinsson 1971). However, the present study shows that in the *C. humilis* complex no close relationship exists among the taxa with soredia similar in size. *C. nashii*, *C. humilis* and *C. conista,* for example, produce farinose soredia (< 30 μm) but do not form a monophyletic group.

Cortex features (presence or absence of cortex, surface roughness) are often used as taxonomic characters in *Cladonia* (Ahti 1980; Hammer 1991, 1995). Yet we find taxa with similar cortex which are not closely related, while specimens whose cortex characteristics are very different appear lumped together within the same clade. For example, clade A contains samples without cortex, with areolate cortex and with smooth and continuous cortex. It is worth noting the case of *C. kurokawae*, with a cortex more or less smooth and continuous, and *C. subconistea*, with areolate verruculose cortex; they turned out to be conspecific. The differences in the cortex type may represent different development stages, since in some species of *Cladonia* it has been found that cortex characteristics vary during development. One of the many examples that we can quote is *C. cinerella* Ahti which has a smooth cortex in early development stages, but soon becomes areolate (Ahti 2000). It should be also noted that in general many of these characters are very difficult to interpret correctly in herbarium specimens, because the environmental conditions in different vegetation communities can strongly affect their phenotype.

Though the primary thallus in general is not much used for species delimitation due to the paucity of recognizable, constant taxonomic characters, in the case of *C. cyathomorpha* the presence of veins on the largish squamules can be used as a taxonomic character to distinguish the species.

In the *C. humilis* complex, secondary metabolites have been attributed a great relevance to differentiate taxa. However, this study we found that most of the clades have more than one chemotype. Bourgeanic acid, so far restricted to *C. conista* and *C. pulvinella*, appears also, though inconstantly, in *C. hammeri*. Presence or absence of fatty acids were considered likewise taxonomically significant, and in fact it is proved that, in some lichenized fungi, phylogenetic lineages correlate with the presence of fatty acids (Spribille et al. 2011). However, in other species of *Cladonia* the presence of the fatty protolichesterinic acid is inconstant at in one monophyletic lineage (Pino-Bodas et al. 2011b). We have also found that atranorin and psoromic acid are inconstant in several lineages (A, C, E, G). As a matter of fact, it is frequent in *Cladonia* that these two substances inconstantly appear in numerous species (for example, atranorin in *C. pyxidata*, *C. furcata*, *C. scabriuscula* etc.). Phylogenetic studies are proving that secondary metabolites in *Cladonia* have less taxonomic value than thought, as studies on several chemically polymorphic lineages have demonstrated (Lendemer & Hodkinson 2009; Piercey-Normore et al. 2010; Pino-Bodas et al. 2011). However, each case must be individually evaluated.

Conclusions

The hypothesis posed at the beginning of the study that species with distribution restricted to the Mediterranean climate in both Europe and North America, can be genetically different owing to the great geographic distance, is true in the case of *C. hammeri.* However, this hypothesis could not be proved for *C. pulvinella,* since material from North America was not available.

The use of multiple loci in species delimitation allows a more realistic vision of the evolutionary history of the group under study. In addition, the use of multiple statistical procedures and tools, such as GSI, F_{ST} or the study of intra- and inter-specific genetic distances, is convenient to obtain a stronger support in order to draw conclusions about species boundaries.

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Molecular data do not support current circumscription of *Cladonia furcata* and *C. subrangiformis* (Cladoniaceae)

VIII

ARTÍCULO VIII

Molecular data do not support current circumscription of *Cladonia furcata* and *C. subrangiformis* (Cladoniaceae)

Raquel Pino-Bodas, Ana Rosa Burgaz, María P. Martín & H. Thorsten Lumbsch

Manuscrito Inédito

Cladonia furcata y *C. subrangiformis* son dos especies estrechamente relacionadas cuyo estatus taxonómico ha sido muy controvertido. Se caracterizan por tener un talo primario evanescente y un talo secundario formado por podecios subulados, corticados y que se ramifican de forma dicótoma. Mientras que *C. furcata* tiene una amplia distribución, estando presente en todos los continentes, *C. subrangiformis* se restringe a Europa. Los caracteres utilizados para distinguirlas han sido la presencia de atranorina en *C. subrangiformis,* el tipo de ramificaciones y el ángulo de estas. *Cladonia furcata* es morfológicamente muy variable, lo que condujo a que se describieran varios taxones infraspecíficos; para algunos autores *C. subrangiformis* era una subespecie de *C. furcata*. En el presente trabajo se investigaron las relaciones entre estos taxones usando secuencias de DNA de tres loci (ITS rDNA, *rpb2*, IGS rDNA) y 22 caracteres, morfológicos y químicos. Se emplearon métodos de reconstrucción filogenética basados en máxima parsimonia, máxima verosimilitud e inferencia bayesiana. También se emplearon redes de haplotipos. Se incluyen en el estudio otras las especies relacionadas, *C. farinacea*, *C. stereoclada* y *C. scabriuscula,* la mayoría de ellas incluidas en algún momento como taxones infraspecíficos de *C. furcata.* Los análisis de máxima parsimonia, máxima verosimilitud y bayesianos de la matriz de datos combinada revelaron la existencia de dos clados bien apoyados. Sin embargo, ninguno de ellos corresponde a ninguna de las especies estudiadas. Ni *C. furcata* ni *C. subrangiformis* son monofiléticas. Tampoco se encontró ningún carácter, ni morfológico ni químico, que pudiese distinguir por sí mismo dichos linajes. Ninguno de los quimiótipos de *C. subrangiformis* formó un grupo monofilético.

Molecular data do not support current circumscription of *Cladonia furcata* and *C. subrangiformis* (Cladoniaceae)

Raquel Pino-Bodas[1], Ana R. Burgaz[1], María P. Martín[2] and H. Thorsten Lumbsch[3]

1 Departamento Biología Vegetal 1, Facultad de Biología, Universidad Complutense de Madrid, E-28040 Madrid, Spain; 2 Departamento de Micología, Real Jardín Botánico, RJB-CSIC, E-28014 Madrid, Spain; 3 Department of Botany, The Field Museum, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA

Abstract: *Cladonia furcata* and *C. subrangiformis* are two closely related taxa whose taxonomic status has raised some controversy. In the present paper, the relationships between these taxa are investigated using three molecular markers (ITS rDNA, *rpb2*, IGS rDNA sequences), as well as 22 phenotypical characters. Other related species included in this study are *C. farinacea*, *C. stereoclada* and *C. scabriuscula*. Maximum parsimony, maximum likelihood and Bayesian analyses of combined dataset reveal the existence of two well supported clades. No morphological or chemical feature has been found which could distinguish by itself these lineages. Neither *C. furcata* nor *C. subrangiformis* are monophyletic. None of the chemotypes in *C. subrangiformis* form a monophyletic group.

Keywords: phylogeny, *Cladonia farinacea,* secondary metabolites.

Introduction

The taxa *Cladonia furcata* (Huds.) Schrad. and *C. subrangiformis* Sandst. were formerly included in the *Ascyphiferae* Tornabete section of the genus *Cladonia* (Ahti 2000). This section consisted of species characterized by an evanescent primary thallus, generally subulate podetia with dichotomous branching type and axils closed when young, later laterally open; with corticate surface and usually without soredia. In these species, atranorin and fumarprotocetraric acid are the most frequent secondary metabolites (Ahti 2000). Using DNA sequences, Stenroos et al. (2002) showed that the *Ascyphiferae* section is polyphyletic, but *C. furcata*, *C. scabriuscula* (Delise) Nyl. and *C. farinacea* (Vain.) A. Evans, within the same section, formed a monophyletic group (*C. subrangiformis* was not included in Stenroos' study).

Cladonia furcata is morphologically very variable, which led several authors to distinguish many infraspecific taxa (Vainio 1887; Fink 1904; Thomsom 1968; Egan 1972). Nevertheless, most authors currently consider the morphological variation of *C. furcata* to be an effect of phenotypical plasticity (Ahti 1977), or to represent development stages of this species (Jahns & Beltman 1973; Jahns et al. 1978). *Cladonia subrangiformis* is morphologically so similar to *C. furcata* that it is often difficult to tell them apart. Since the beginning of the 20th century with the description of *C. subrangiformis* (Sandstede 1922) lichenologists have shown great interest in the relationships between these, as well as in the morphological variability

of both taxa; indicated by the considerable amount of literature on the subject (Asahina 1942; Evans 1954; Ullrich 1956; Schade 1964, 1966; Hennipman 1967; Pišut & Wagner 1973; Paus 1997). Some authors have considered *C. subrangiformis* as having infraspecific rank within *C. furcata* (Wirth 1995; Des Abbayes 1937; Schade 1966; Haswksworh 1969; Hennipman 1967; Verseghy, 1975; Clauzade & Roux 1985; James 2009). Others attribute the species rank to both of them (Nimis 1993; Burgaz & Ahti 2009).

Cladonia furcata and *C. subrangiformis* are distinguished by the presence of atranorin (K+) in *C. subrangiformis* (Sandstede 1922), while it is generally lacking in *C. furcata* (K−), although in certain areas *C. furcata* specimens containing atranorin have been found (Huovinen et al. 1990; Etayo & Burgaz 1997; Ahti 2000; Huneck et al. 2004). Moreover, these two taxa can be morphologically distinguished by the branching type (van den Boom 2002) and by the branching angle, acute in *C. furcata,* while usually right (James 2009) or obtuse in *C. subrangiformis.* Podetia tend to be erect in *C. furcata,* while in *C. subrangiformis* they usually are prostrate and tougher (Wirth 1995). Podetial surface is generally wrinkled, provided with many short, thorny branches (Des Abbayes 1937), while it is smoothly corticate and lacks thorny branching in *C. furcata.* From an anatomical viewpoint Ahti (2000) pointed out the presence of a striate stereome in *C. furcata. Cladonia furcata* is found on acidic substrata, while *C. subrangiformis* grows on calcareous substrata (Nimis 1993; van den Boom 2002; James 2009; Paul et al. 2009). Paus (1997) found that *C. furcata* grows on substrata with high contents in humus and acidic pH, while the substrata where *C. subrangiformis* develops have a lesser humus contents and a higher pH.

A detailed revision of *C. furcata* and *C. subrangiformis* was carried out by Günzl (2004). The author concluded that most morphological characters show a strong dependence on light and humidity. She also studied the variation of ITS nrDNA region in *C. furcata* and *C. subrangiformis* specimens from Germany. Günzl did not find any correlation between morphology and DNA sequence variation, concluding that both taxa should be considered as phenotypical variants of one single species. However, unlike *C. furcata,* which is cosmopolitan, *C. subrangiformis* has its distribution center in the Mediterranean Region (Ahti & Shorabi 2006), where it presents the higher morphological and chemical variability. Burgaz & Ahti (2009) found five different chemotypes for this taxon: 1) atranorin and fumarprotocetraric acid, 2) atranorin, fumarprotocetraric acid, physodalic and hypoprotocetraric acids, 3) atranorin and psoromic acid, 4) psoromic and conpsoromic acids, 5) atranorin and bourgeanic acid, with the inconstant presence of physodalic or fumarprotocetraric acids. Though a specimen of *C. furcata* containing psoromic acid was mentioned (Barendregt et al. 1982), Burgaz et al. (1999) concluded that this was a sample of *C. subrangiformis*.

The aim of this work is to address the taxonomical status of *C. furcata* and *C. subrangiformis.* For this purpose we analyzed phenotypical features and sequences of three loci in *C. furcata* and *C. subrangiformis,* including most of the described chemotypes in those species.

Material and Methods

Sampling

This study is based on 805 samples from CANB, FH, H, MA, MACB, L, S and UPS herbaria corresponding to *C. furcata*, *C. subrangiformis*, *C. scabriuscula*, *C. farinacea* and *C. stereoclada* Abbayes. We tried resuming all morphological and

chemical variation in a group of 66 samples selected for molecular analyses. Samples of *C. corsicana* (Rondon and Vězda) Pino-Bodas, Burgaz and M. P. Martín, *C. farinacea*, *C. humilis* (With.) J. R. Laundon, *C. scabriuscula*, and *C. stereoclada* were included because these species were shown to be closely related to *C. furcata* and *C. subrangiformis* (Stenroos et al. 2002; Pino-Bodas et al. 2012). *Cladonia turgida* Hoffm. and *C. gracilis* subsp. *gracilis* L. were used as outgroups based on their emplacement in our own unpublished phylogenetic analyses. The specimens of *C. furcata* and *C. subrangiformis* were identified using morphological and chemical characters according to Wirth (1995), Scholz (2000) and Burgaz & Ahti (2009).

Chemical composition was studied by TLC according to the standardized procedures of White & James (1985), with solvent systems A and B.

Table 1. Samples used in the molecular study with GenBank accession numbers.

Sample	Collection	Nº GenBank ITS rDNA	*rpb2*	IGS rDNA
C. furcata 1	Spain, León, *A. R. Burgaz* (MACB 91055)	+	+	+
C. furcata 2	Spain, Segovia *A. R. Burgaz* (MACB 93519)	+	+	+
C. furcata 3	Spain, Lugo *A. R. Burgaz* (MACB 92559)	+	+	+
C. furcata 4	Portugal, Alto Alentejo, *A. R. Burgaz* (MACB 91087)	+	+	+
C. furcata 5	USA, Virginia, *J. C. Lendemer* (FH 239444)	+	+	+
C. furcata 6	Denmark, Syddanmark, *E. S. Hansen* (H)	+	+	+
C. furcata 7	Denmark, Capital Region, *E. S. Hansen & J. Hansen* (H)	+	+	+
C. furcata 8	Portugal, Madeira, *H. Väre* (H)	+	+	+
C. furcata 9	Finland, Päijänne Tavastia, *V. Haikonen* (H)	+	+	+
C. furcata 10	Finland, Central Finland, *T. Rintanen* (H)	+	+	+
C. furcata 11	Finland, Åland Islands, *M. Sternberg* (H)	+	+	+
C. furcata 12	Italy, Sardegna (H)	+	+	+
C. furcata 13	Croatia, Dubrovnik-Neretva, *A. R. Burgaz* (MACB 101098)	+	+	+
C. furcata 14	Spain, Menorca, *A. R. Burgaz* (MACB 96253)	+	+	+
C. subrangiformis 1	Spain, Castellón, *A. R. Burgaz* (MACB 91011)	+	+	+
C. subrangiformis 2	Spain, Soria, *A. R. Burgaz* (MACB 91155)	+	+	+
C. subrangiformis 3	Spain, Soria, *A. R. Burgaz* (MACB 102468)	+	+	+
C. subrangiformis 4	Spain, Asturias, *A. R. Burgaz* (MACB 102466)	+	+	+
C. subrangiformis 5	Portugal, Beira Alta, *A. R. Burgaz* (MACB 102469)	+	+	+
C. subrangiformis 6	Spain, Madrid, *A. R. Burgaz* (MACB 102467)	+	+	+
C. subrangiformis 7	Bosnia I Herzegovina, Sarajevo, *A. R. Burgaz* (MACB 101105)	+	+	+
C. farinacea 1	USA, Pensylvania, *J. C. Lendemer* (H)	+	+	+
C. farinacea 2	Chile, Region XII, *A. R. Burgaz* (MACB 920789	+	+	+
C. stereoclada 1	Spain, Canary Islands, *A. R. Burgaz* (MACB 97913)	+	+	+
C. stereoclada 2	Spain, Canary Islands, *A. R. Burgaz* (MACB 97911)	+	+	+
C. scabriuscula	Chile, Region XII, *A. R. Burgaz* (MACB 91976)	+	+	+
C. corymbescens	Thailand, *S. Parnmem* (H)	+	+	+
*C. corsicana*1	Spain, Sevilla, *A. R. Burgaz* (MACB 100763)	JF288797	JF288833	+
C. corsicana 2	Spain, Sevilla, *A. R. Burgaz* (MACB 101074)	JF288798	JF288834	+
C. corsicana 3	Portugal, Algarve, *A. R. Burgaz* (MACB 101073)	JF288799	JF288835	+
C. corsicana 4	Spain, Cádiz, *A. R. Burgaz* (MACB 100765)	JF288800	JF288837	+
C. humilis 1	Spain, Toledo, *A. R. Burgaz* (MACB 92807)	JF926622	JF926585	+
C. humilis 2	Portugal, Baixo Alentejo, *A. R. Burgaz* (MACB 97326)	JF926626	JF926587	+
C. humilis 3	Portugal, Madeira, *P. Alanko* (H)	JF926616	JF926582	+
C. humilis 4	USA, California, *K. Knudsen* (H)	JF926618	JF926578	+
C. turgida 1	Canada, Newfoundland, *C. Lendemer* (H)	JF288801	+	+
C. turgida 2	Canada, Otario, *J. C. Lendemer* (H)	JF288802	+	+
C. gracilis	Spain, Palencia, *A. R. Burgaz* (MACB 94216)	JN811386	JN811412	JN811354

Molecular work

Previous to DNA isolation, the secondary metabolites were extracted by soaking the samples in acetone for two hours, and the liquid was used for thin layer chromatography (TLC). The DNeasy Plant Mini Kit (Quiagen) was used to extract DNA, according to the manufacturer's instructions. The DNA was dissolved in 200 µl of buffer included in the kit. The three following loci were amplified: nuclear ITS rDNA using primer ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) or ITS1/ITS2 (White et al. 1990) and ITS3/ITS4, *rpb2* using two pairs of primers, RPB2-5F/RPB2-7R (Liu et al. 1999) and RPB2dRaq/RPB2rRaq (Pino-Bodas et al. 2010), and IGS using IGSf/IGSr (Wirtz et al. 2008). PCRs were carried out with Ready-to-Go-PCR Beads (GE Healthcare Life Sciences, UK). The volume of reaction was 25 µl for each tube, with 0.4 mM final concentration of primers. The amplification programs were: 1) 94ºC for 5 min; 5 cycles of 94ºC for 30 s, 54ºC for 30 s and 72 ºC for 1 min; and 33 cycles of 94ºC for 30 s, 48ºC for 30 s and 72ºC for 1 min; with a final extension of 72ºC for 10 min for nuclear ITS rDNA, 2) initial denaturation at 94ºC for 5 min; 40 cycles of 95ºC for 1 min, 52ºC for 30 s and 72ºC for 2 min; with a final extension at 72ºC for 10 min for *rpb2* and IGS rDNA loci. PCR products were purified using the QIAquick gel extraction Kit (QIAGEN, Hilden, Germany) or ExoSAP-IT (USB Corporation, OH, USA). The sequencing reactions were done at Macrogen (South Korea) service (www.macrogen.com), with the same primers used for the PCR. The samples that yielded uninterpretable sequences were cloned into pGEM T vector (Promega).

Sequence alignment and phylogenetic analyses

The alignments were made manually with SE-AL v2.0a11 (Rambaut 2002) for each locus separately. Six ambiguous positions in the ITS nrDNA matrix were removed, while the matrixes of IGS rnDNA and *rpb2* did not contain ambiguous positions. Each region was analyzed by maximum parsimony (MP) and maximum likehood (ML). MP analyses were made using PAUP version 4.0.b.10 (Swofford 2002), using heuristic searches with 1000 random taxon-addition replicates with TBR branch swapping and MulTrees option in effect, equally weighted characters and gaps treated as missing data. Bootstrap analysis was used with 1000 replicates and the heuristic option. The ML analyses were implemented using Garli v0.96 (Zwickl 2006) assuming a GTR+I+G model. Congruence among the different topologies inferred from the loci was tested following Lutzoni et al. (2004). The independent analyses of each locus showed two incongruences, which were excluded and the datasets were combined. MrModeltest (Nylander 2004) was used for selecting the most appropriate nucleotide substitution model for each locus using the AIC criterion. The combined dataset was analyzed by MP, ML and a Bayesian approach. The Bayesian analysis was carried out using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). The combined dataset was partitioned in five partitions: ITS rDNA, IGS rDNA and each of three codon positions of *rpb2*, respectively. The model GTR+G was applied to each partition of *rpb2,* while the SYM+I model was used for ITS rDNA and HKY+I was applied to IGS rDNA. The posterior probabilities were approximated by sampling trees using Markov Chain Monte Carlo (MCMC). The posterior probabilities of each branch were calculated by counting the frequency of trees visited during MCMC analysis. Two simultaneous runs with 20,000,000 generations each starting with a random tree and employing 4 simultaneous chains were executed. Every 1000th tree was saved into a file. The first 1,000,000

generations (i.e. the first 1000 trees) were deleted as the "burn in" of the chain. AWTY (Nylander et al. 2008) was used to determine when the chains reached the stationary stage. The 50% majority-rule consensus tree was calculated using the "sumt" command of MrBayes.

Haplotypes networks under statistical parsimony with a confidential interval of 95% were generated with TCS 1.21 (Clement *et al*. 2000) for each locus (ITS rDNA, IGS rDNA and *rpb2*) including only the samples of *C. furcata* group. Gaps were coded as missing data.

Hypothesis testing

Tests to assess whether our data significantly rejected the monophyly of described species were done using two tests, the Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa 1999) and the expected likelihood weight (ELW) test (Strimmer & Rambaut 2002). Both were implemented in Tree-PUZZLE 5.2 (Schmidt et al. 2002) using the combined dataset. Four constrained trees representing the hypotheses 1) Monophyly of *C. furcata*, 2) Monophyly of *C. subrangiformis* 3) Monophyly of *C. farinacea* and 4) Monophyly of *C. furcata* group (Table 2) were tested.

Morphological and chemical studies

Measurements of phenotypical characters were taken to characterize the morphology of each species and to test whether morphological differences exist among the clades. The continuous characters studied included: length, width and thickness of the podetia, thickness of the cortex, the medulla and the stereome, diameter of the central canal, angle of the branches and number of ramifications. The qualitative characters examined were: open or close axiles, presence or absence of white medullary spots on the older parts of the podetia, presence or absence of squamules on the podetia, smooth or striate stereome, smooth or rimose cortical surface and presence or absence of spinuliform proliferations (Fig 1).

The Kolmogorov-Simirnov test was used to check normality and Levence statistic to check the homogeneous variance in the continuous characters. These characters were analyzed by principal component analysis (PCA) on correlation matrix, using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). The analysis was made again excluding the variable "length of the podetia", because the communalities value for this variable was < 0.5.

Results

Phylogenetic analyses

Ninety three new sequences were generated for this study (ITS: 27, *rpb2*: 29, IGS: 37; Table 1). The combined dataset contained 1871 characters, 607 of which corresponded to ITS, 891 to *rpb2* and 373 to IGS nrDNA. 1530 characters were constant and 190 were parsimony informative (64 in the ITS rDNA dataset, 40 in the IGS rDNA and 86 in the *rpb2*). The MP analyses yielded 1000 equally parsimonious trees, 474 steps long, with a value CI = 0.798 and RI = 0.889. ML analysis yielded a tree with a likelihood value of LnL = -5494.84 while the Bayesian analysis gave LnL = -5542.10. The other parameters of the Bayesian analysis were: r(A-C) = 0.09009 (0.000284), r(A-G) = 0.259815 (0.000802), r(A-T) = 0.080507 (0.000253), r(C-G) = 0.052404 (0.000143),

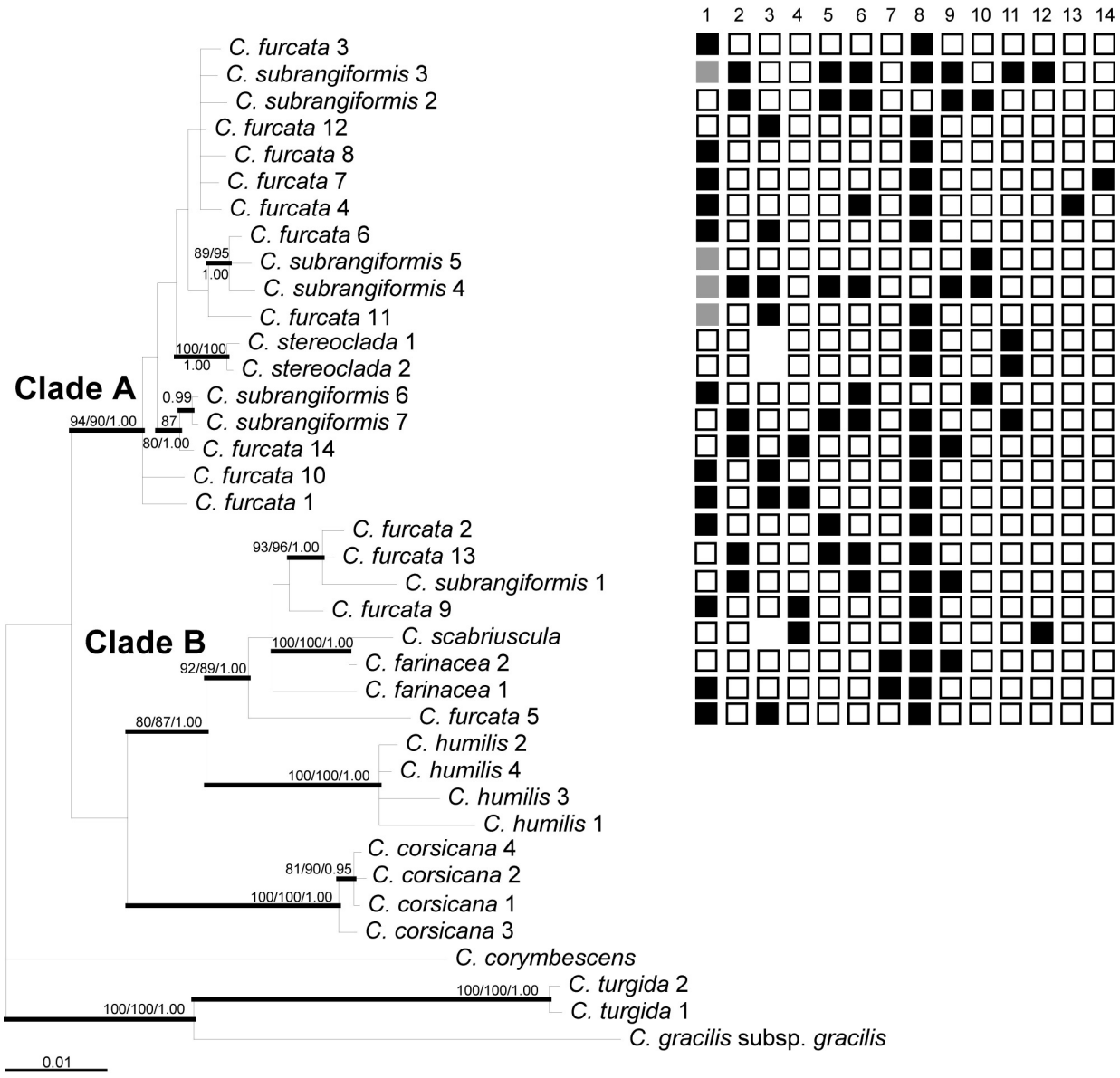

Fig 1. The 50% Majority Rule Bayesian tree based on a combined data set including ITS rDNA, IGS rDNA and *rpb2*. Branches supported with posterior probability ≥ 0.95 and bootstrap > 70% are indicated in bold. Bootstrap value > 70% for MP/Bootstrap value > 70% for ML/posterior probability >0.95 for Bayesian analysis at branches. The character states observed in each specimen are indicated. 1) axiles □ closed, ■ open, ■ both; 2) white medullary spot □ ausence, ■ presence; 3) estereome structure □ smooth, ■ striated; 4) presence of squamules on the podetia □ ausence, ■ presence; 5) podetial surface □ smooth, ■ rimose; 6) spiuniforms branches □ ausence, ■ presence; 7) Soredia □ ausence, ■ presence; 8) Fumarpotocetraric acid □ ausence, ■ presence; 9) atranorin □ ausence, ■ presence; 10) psoromic acid □ ausence, ■ presence; 11) bourgeanic acid □ ausence, ■ presence; 12) zeorin □ ausence, ■ presence; 13) physodalic acid □ ausence, ■ presence; 14) hypoprotocetraric acid □ ausence, ■ presence.

r(C-T) = 0.437015 (0.001070), r(G-T) = 0.071250 (0.000203), κ = 5.878593 (1.655081), α = 0.235590 (0.003483) and pinvar = 0.630692 (0.001040).

The three analyses generated topologically identical trees and thus only the Bayesian tree is shown here (Fig. 1). The samples of *C. furcata* and *C. subrangiformis* fall into two well supported, monophyletic clades. Clade A contains 10 samples of *C. furcata*, 6 of *C. subrangiformis* and 2 of *C. stereoclada,* all from Europe; with *C. stereoclada* samples highly supported as monophyletic group nested within this group. Clade A is a sister group of a clade formed by 4 samples of *C. corsicana*, 4 of *C. humilis,* and the clade B which contains 2 samples of *C. farinacea* from Chile and USA*,* 4 of *C. furcata* from Europe and USA, 1 of *C. subrangiformis* from Spain and 1 of *C. scabriuscula* from Chile. Clade B is closely related with *C. humilis*. As shown in Figure 1, both clades are morphologically heterogeneous. Clade A contains samples with fumarprotocetraric acid, atranorin, psoromic acid and bourgeanic acid. Clade B only contains samples with fumarprotocetraric acid alone, or with atranorin. *Cladonia corsicana* formed a monophyletic group, but its relationships with the other taxa included in the analyses gained only low support.

In the network analyses, the ITS sequences represented 25 haplotypes combined in a single network (Fig 2). *Cladonia furcata* contained 14 haplotypes (56%), *C. subrangiformis* 7 (28%), *C. stereoclada* 2 (8%), *C. farinacea* 2 (8%) and *C. scabriuscula* 1 (4%). The haplotype diversity was high, 26 specimens in 25 haplotypes, with 25 uniques haplotypes and 1 haplotype shared with 2 samples (*C. subrangiformis* 4 and *C. furcata* 4). In general, the samples contained in clade B were separated by more subtitutions. The network contained secondary connections. The IGS rDNA sequences yielded 20 haplotypes in a single network (Fig 2). The most common haplotype was H1, which was exclusively made up of *C. furcata* specimens. No haplotypes were shared between *C. furcata* and *C. subrangiformis*. *Cladonia furcata* contained 10 haplotypes (50%), *C. subrangifromis* 6 (30%), *C. stereoclada* 1 (5%), *C. farinacea* 2 (10%) and *C. scabriuscula* 1 (5%). Nine haplotypes were detected in the *rpb2* data set distributed in 3 unconnected networks (Fig 2). The haplotype distribution was highly congruent with the results yielded in the phylogenetic analyses of combined dataset. Network 1 contained all samples of clade A distributed into 3 haplotypes. The most frequent haplotype (H1) was shared by samples of *C. furcata*, *C. subrangiformis* and *C. stereoclada*. The other two haplotypes corresponded to *C. furcata* (H3) and *C. subrangiformis* (H2), respectively. The samples of clade B were allocated among two networks: network 2, with 5 haplotypes (2 haplotypes of *C. furcata*, 1 of *C. subrangiformis* and 2 of *C. farinacea;* one haplotype of *C. farinacea* was shared with *C. scabriuscula***)** and network 3 with only one haplotype (*C. furcata* 5).

Two alternative hypotheses, the monophyly of *C. furcata* and the monophyly of *C. subrangiformis,* were tested (Table 2) by SH and ELW tests, and both of them were rejected significantly. The monophyly of *C. farinacea* and the monophyly of *C. furcata* group were rejected by SH test, but not by ELW.

Table 2 Results of Shimodaira–Hasegawa and Expected Likelihood Weight topology tests. * denote significant results.

	Ln L	SH	ELW
C. subrangiformis monophyletic	-5852.21	0.0000*	0.0000*
C. furcata monophyletic	-6481.68	0.0000*	0.0000*
C. farinacea monophyletic	-5688.25	0.1820	0.0043*
C. furcata group monophyletic	-5681.35	0.2430	0.0185*

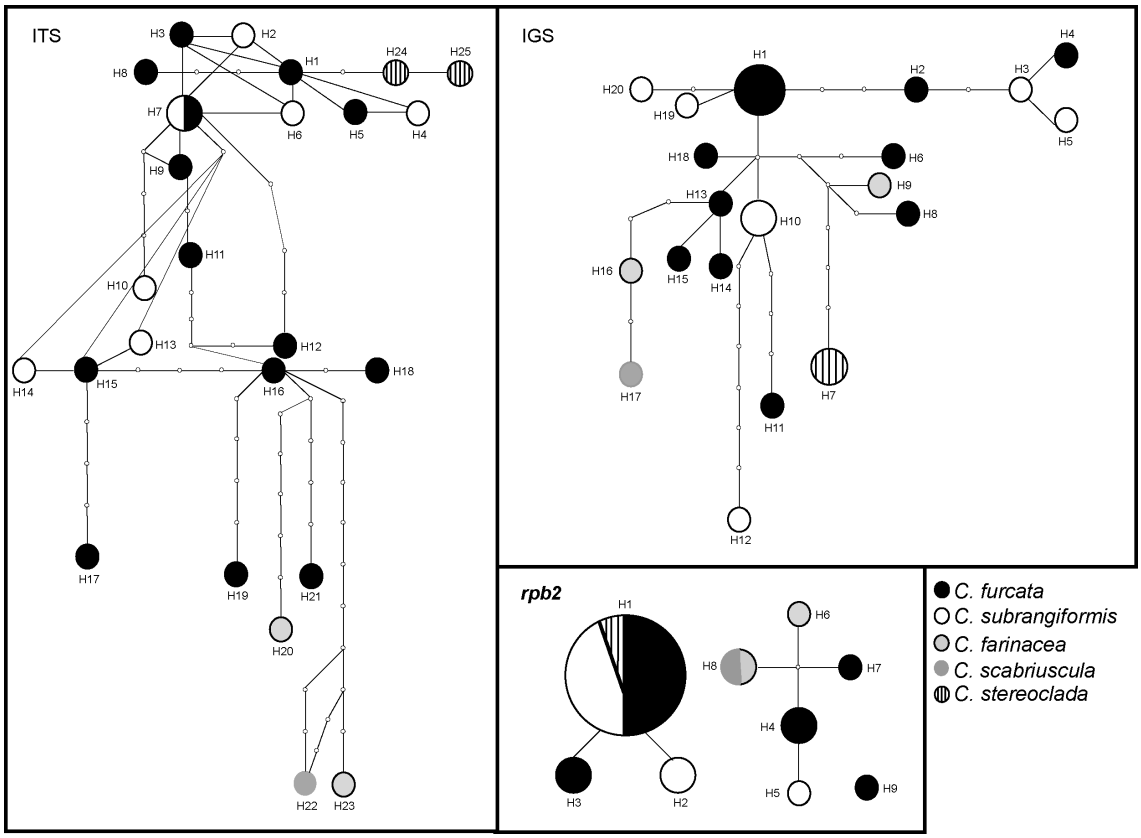

Fig 2. Networks for the species of *Cladonia furcata* group under 95% parsimony criterion of ITS rDNA, IGS rDNA and *rpb2*. The circles represent the haplotypes, the size is proportional to haplotype frequency. Conecting lines indicate a single subtitutions and the small circles represent missing haplotypes.

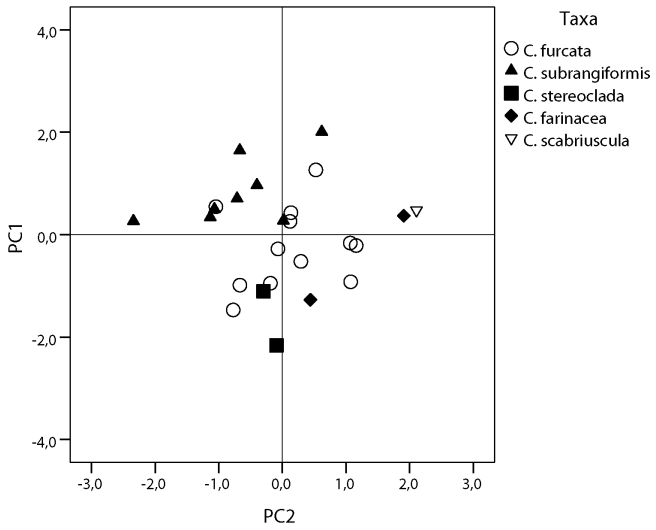

Fig 3. Principal component analysis (PCA) based on eight continous variables.

Multivariate morphological analysis

The PCA analysis (Fig 3) summarized the 68.84% of the total variance (48.88% and 19.97% accounted for component 1 (PC1) and component 2 (PC2), respectively. In the PC1, the characters with most influence were thickness of the podetia, thickness of the cortex, thickness of the medulla, angle of the branches and diameter of the central canal, whereas in the PC2 the most influential character was the thickness of the stereome. There is a degree of overlapping among the species, but *C. stereoclada* is separated. *Cladonia furcata* and *C. subrangiformis* were partially separated in two groups; *C. farinacea* and *C. scabriuscula* form overlapping clusters with *C. furcata* and *C. subrangiformis.*

Discussion

The independence of *Cladonia furcata* and *C. subrangiformis* has always been questioned due to the existence of numerous specimens with intermediate morphology. Des Abbayes (1937) documented and described numerous intermediate forms between both taxa. The author concluded that *C. subrangiformis* should be considered as a mere variant of *C. furcata* with the white spots at the podetial base and the development of thorny branches as distinguishing characters. Evans (1954), however, considered that the existence of atranorin as distinguishing character and pointed out that Sandstede (1931) stated that the morphological characters were responses to unfavourable ecological conditions. Our morphometric analyses suggest there is in fact a certain morphological difference among the currently circumscribed *C. furcata*, *C. subrangiformis* and *C. stereoclada* (Fig 3), although the groups partially overlap due to the high morphological plasticity. However, molecular data are not congruent with either morphology or chemistry, since neither morphologically similar samples, nor chemically identical ones form monophyletic groups. Neither *C. furcata* nor *C. subrangiformis* are monophyletic groups in our study. Instead two well supported clades, grouping the specimens of *C. furcata*, *C. subrangiformis* and allied species, were found, but none of the studied phenotypical characters characterize those clades phenotypically (Fig 1). Clade A as well as clade B includes specimens with thorny branches, opened or closed axils, with or without white spots at the podetial base, with smooth or striate stereome. Both clades also include specimens with or without atranorin. However, samples containing psoromic and bourgeanic acids only fall into clade A . In *C. subrangiformis* specimens with psoromic acid appear in clade A intermingled with specimens containing bourgeanic acid or atranorin and with specimens of *C. furcata* that only contain fumarprotocetraric acid.

The haplotype networks neither showed a pattern corresponding to the current species described. This fact, along with the results of the hypothesis testing which discard the monophyly of both taxa, leads us to reject the hypothesis that *C. furcata* and *C. subrangiformis* are two independent species, so supporting the conclusion reached by Günzl (2004), based on the study of the commonest chemotypes: the one that contains only fumarprotocetraric acid and the one which contains fumarprotocetraric acid along with atranorin.

Since specimens of both taxa appear in the two clades mixing with the specimens of other related species, the taxonomical problem can not be solved in a easy way, such as by synonymizing *C. subrangiformis* with *C. furcata.* The distribution of the OUT's in the phylogenetic tree shown in figure 1 could be indicating either that the speciation is recent and the species still keep ancestral

polymorphisms, or that there are more than one phylogenetic species in the group *C. furcata*-*C. subrangiformis*. However, the alternative hypothesis, monophyly of *C. furcata* group (including *C. furcata, C. farinacea, C. scabriuscula, C. subrangiformis* and *C. stereocalda)* was rejected only by SH test. This topology was yielded by the locus *rpb2*, although the ITS rDNA network showed that the samples of clade B frequently were separated by more mutational steps. We consider that the current data are not enough to clarify the taxonomy of *C. furcata* group. Further studies of more independent loci will be necessary for this. As in the phylogenetic analysis of Stenroos et al. (2002), *C. furcata* group (clade B) appears to be phylogenetically related to *C. humilis;* however we consider it premature to hold this relation as sure, since the genus *Cladonia* comprises about 450 species, while the sampling done is rather limited and, in addition, these taxa are morphologically very disparate.

Stenroos et al. (2002) pointed out that some taxonomical problems with widely distributed taxa should be expected. Regional morphological variations of *C. furcata* (Hammer 1995; Ahti 2000) have been found, what could indicate that it does not constitute a unique species. However, it will be necessary to count on a great deal of specimens coming from all the continents to confirm this hypothesis.

The lack of resolution within the clades does not permit us to determine unambiguously the number of phylogenetic species in this group. The only taxon which appears as monophyletic is *C. stereoclada*, within clade A. This species is a Macaronesian endemism (Pišút 2009). The morphological character that distinguishes it from *C. furcata* is the presence of narrow podetia, either solid or with a very thin central cavity (Des Abbayes 1937). The presence of solid podetia in *Cladonia* is very rare; only another species is known to present this character, C. solida Vainio (Ahti 2000). Fumarprotocetraric acid was the only chemical substance quoted for this species; however, two of the specimens studied here contained bourgeanic acid along with fumarprotocetraric. In the separate analyses of ITS rDNA and IGS rDNA regions (not shown), the samples of *C. stereoclada* clustered into a monophyletic clade which included samples containing only fumarprotocetraric acid and samples containing bourgeanic and fumarprotocetraric acids. Therefore, the species *C. stereoclada* can have two chemotypes.

Cladonia farinacea turned out not to be monophyletic, a similar result to that found by Stenroos et al. (2002). However, the SH test did not reject the monophyly of this taxon. The species grows in Argentina and Chile (Stenroos et al. 1992), in North America (Thomson 1968) and Asia (Asahina 1974; Ahti 1992). It has been questioned that the samples of C. farinacea from South and North America belonged to the same species (Huovinen et al. 1990; Ahti 1992). The material coming from South America contains atranorin, while the samples from North America lack this substance (Huovinen et al. 1990). The number of samples included in this paper was too limited to permit us to clarify this taxonomical question. A molecular and morphological study with a high number of samples from both regions is necessary to solve this problem.

According to the previous hypoteses, based in the study of ITS rDNA region (Pino-Bodas et al. 2011), *C. corsicana* belonged to a clade where the species of *C. furcata* group were included; in our phylogenetic studies, however, the phylogenetic position of this taxon remains unsolved. Further phylogenetic studies, involving more taxa, are necessary to solve the relationships of this taxon.

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Species delimitation in *Cladonia* (Ascomycota): a challenge to the barcoding philosophy

IX

ARTÍCULO IX

Species delimitation in *Cladonia* (Ascomycota): a challenge to the barcoding philosophy

Raquel Pino-Bodas, María P. Martín, Ana Rosa Burgaz & H. Thorsten Lumbsch

Molecular Ecology Resource (2012) En revisión

El género de hongos liquenizados *Cladonia* está muy diversificado, conteniendo cerca de 500 especies. El código de barras de DNA (barcode) aceptado para hongos (ITS rDNA) con frecuencia falla en las identificaciones de *Cladonia*. Con objeto de hallar otros marcadores, que en combinación con la región ITS rDNA puedan ser utilizados para la identificación de especies en *Cladonia,* se estudiaron los loci IGS rDNA, *ef1α*, *rpb2* y *cox1*. Se analizaron 784 secuencias de 36 especies. La tasa de éxito de amplificación por PCR, la variación de las distancias genéticas intraespecíficas e interespecíficas calculadas mediante el modelo K2P, así como el porcentaje de identificación correcta (PCI), se tuvieron en cuenta para evaluar cada uno de los posibles barcodes. El marcador que presentó un rango menor de distancias genéticas intraespecíficas fue *cox1,* seguido de ITS rDNA y *ef1α.* De los cinco marcadores estudiados, solo *cox1* presentó un "barcoding gap" (diferencia entre la media de las distancias interespecíficas superior a 10 veces la media de las distancias intraespecíficas). El locus *rpb2* fue el que mostró valores más altos de PCI, pero es el más difícil de amplificar.

Species delimitation in *Cladonia* (Ascomycota): a challenge to the DNA barcoding philosophy

Raquel Pino-Bodas[1], María P. Martín[2], Ana R. Burgaz[1], H. Thorsten Lumbsch[3]

1 Departamento Biología Vegetal 1, Facultad de Biología, Universidad Complutense de Madrid, E-28040 Madrid, Spain, 2 Departamento de Micología, Real Jardín Botánico, RJB-CSIC, E-28014 Madrid, Spain, 3 Department of Botany, The Field Museum, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA.

Abstract: The lichen-forming fungal genus *Cladonia* is species-rich with approximately 500 described species. The accepted barcode for fungi (ITS rDNA) often fails in identifying *Cladonia* spp. In order to find other markers that, in combination with the ITS rDNA region can be used for species identification in *Cladonia*, we studied the loci IGS rDNA, *ef1α*, *rpb2* and *cox1*. A total of 784 sequences from 36 species have been analyzed. PCR amplification success rate, intraspecific and interspecific genetic distance variation, calculated using the K2P model, and the correct identification percentage (PCI) were taken into account to assess possible barcode regions. The marker showing the least intraspecific genetic distance range was *cox1,* followed by ITS rDNA and *ef1α.* Of the five studied markers only *cox1* showed a barcoding gap. The *rpb2* locus showed the highest PCI values, but it was the most difficult to amplify.

***Keywords*:** Monophyletic probability of correct identification; species identification; DNA barcoding; lichenized fungi.

Introduction

DNA barcoding is an important tool for rapid species identification. It is very useful for accelerating biodiversity inventories, managing invasive species, pest controlling and detection of cryptic species. While originally *cytochrome c oxidase I (cox1)* was intended to be a universal DNA barcode marker (Hebert et al. 2003b), subsequently – since the same region can have different evolution rates in the diverse groups and other issues (Britten 1986; Hafner et al. 1994) – separate barcoding markers have been chosen for the three major groups of eukaryotes: plants, fungi, and animals. In animals *cytocrome c oxidase I* (*cox1*) (Hebert et al. 2003a) is used as the standard barcode, while in plants the *matK* and *rbcL* regions (CBOL Plant Working Group 2009) have been chosen for this purpose. The ITS rDNA region has recently been proposed as barcode for fungi (Schoch et al. 2012).

Estimates of the number of fungal species suggest that about 1.5 million fungal species exist (Hawksworth 1991, 2001), of which only about 100,000 species are currently known (Kirk et al. 2008). Although more conservative estimates give a minimal number of 700,000 species (Schmit & Mueller 2007), results from recent environmental sampling projects indicate that the number of fungal species may be much higher than 1.5 million (Blackwell 2011). All these estimates clearly suggest that most of the fungi species are not described as yet. Some of the reasons for the low number of described species include: numerous fungi are microscopic and

therefore inconspicuous; in many cases they show few morphological characters to distinguish among species; and it has been demonstrated that cryptic species are common among different fungal groups (Geiser et al. 1998; Cruse et al. 2002; Pringle et al. 2005; Carriconde et al. 2008; Pavlic et al. 2009). The presence of cryptic species is especially common in lichenized fungi (Crespo & Perez-Ortega 2009; Crespo & Lumbsch 2010; Lumbsch & Leavitt 2011).

The lichen-forming fungal genus *Cladonia* is with 500 described species the largest within Cladoniaceae. Many species complexes exit in this genus, for which the limits among species are not well understood. This is mainly due to the high morphological plasticity of species. While some species are easily recognized phenotypically, the most of taxa are very variable and hence a broad experience and knowledge about the variation of taxa is necessary to identify species within this genus (Ahti 2000), making it extremely difficult to identify species in this ecologically important group of mainly terricolous lichens. This has led to several studies employing molecular data to test current species delimitations in *Cladonia* (e.g., DePriest 1993a, b, 1994; Kotelko & Piercey-Normore 2010; Pino-Bodas et al. 2010a, b, 2011, 2012a, b, c, d). The development of reliable identification tools by means of barcode marker(s) is thus important in the genus *Cladonia.* Some studies, however, have shown that the ITS rDNA region provides a poor resolution for species separation in *Cladonia* (Kotelko & Piercey-Normore 2010; Fontaine et al. 2010; Pino-Bodas et al. 2011). In addition, a study on DNA barcoding in lichenized fungi has demonstrated the lack of a barcoding gap for ITS in the studied *Cladonia* spp. and a high failure percentage in BLAST searches (Kelly et al. 2011). These results prompted us to explore other markers that could be used potentially in combination with the fungal barcode region ITS to reliably identify *Cladonia* spp. Here we test second largest subunit of RNA polymerase II (*rpb2*), translation elongation factor 1 (*ef1α*), nuclear ribosomal intergenic spacer region (IGS rDNA) and cytocrome c oxidase I (*cox1*) as possible barcodes for the identification of *Cladonia* spp. and compare the performance with the ITS region.

Material and Methods

Taxon sampling

This study is based on 148 newly generated sequences and DNA sequences obtained in previous studies in our lab (Pino-Bodas et al. 2010a, b, 2011, 2012a, b, c, d). The taxonomic concept accepted in these papers is adopted here in order to assign each of the sequences to the corresponding species. Here we included 36 *Cladonia* species, 35 of which belong to the supergroup *Cladonia* (Stenroos et al. 2002) and one (*C. cenotea)* that belongs to the supergroup *Perviae*. The number of samples per species varied from 1 to 35, the average being seven samples per species (Table 1S). Most of the samples came from herbaria and were collected between 1918 and 2010. For the ITS rDNA region only the fragment taken as barcode for fungi (Schoch 2012) was considered, i.e. the barcode starts with the last five bases of 18S rDNA (CATTA) and finishes with the first 5 bases of 28S rDNA (AATTG in *Cladonia*).

DNA extraction and amplifications

Total DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The primers used to amplify the different loci are listed in Table 2S. PCRs were carried out with Ready-to-Go-PCR Beads (GE Healthcare Life Sciences, Buckinghamshire, UK) for ITS rDNA, *rpb2*,

ef1α and *cox1*. The volume of each reaction was 25 l, with 0.4 mM final concentration of primers. The amplification of IGS rDNA was carried out using Biotaq polymerase (ECOGEN, Barcelona, Spain) following Pino-Bodas et al. (2012d). PCR products were purified using the QIAquick gel extraction Kit (QIAGEN, Hilden, Germany) or with ExoSAP-IT (USB Corporation, OH, USA). The sequencing reactions were done at Macrogen (South Korea) and assembled using SEQUENCHER^{TM} (Gene Codes Corporation, Inc, Ann Arbor, Michigan, USA).

Analyses

The sequences were aligned using MAFFT (Katoh et al. 2005) for each gene and corrected manually. Genetic distances were calculated in PAUP* 4.0b10 (Swofford 2003) using the Kimura 2-Parameter model (Kimura 1980), which is widely used in DNA barcoding analyses (e.g., Neigel et al. 2007; Ward et al. 2008; Van Velzen et al. 2009, 2012). Histograms of intraspecific and interspecific distances were created for each marker in order to illustrate the amount of overlap of those for each gene (Hebert et al. 2003b). Wilcoxon tests were used to assess the differences in sequence divergence among loci, in combinations of two loci. This test was applied to the samples for which sequences for all four loci studied were available.

Probability of Correct Identification (PCI) was calculated according Suwannasai et al. (2012), using the previous aligments. The p-Distance (also known as "uncorrected distance", the fraction of aligned nucleotide pairs that are not identical pairs) was used to calculate the PCI values. According to this parameter, if a species is correctly identified as monophyletic, it is assumed to be correctly identified. The PCI values vary between 0 and 1. The maximum value (1.0) is reached when all the species are correctly identified. Only samples for which sequences of all four markers were available, were included in these analyses. The Wilson score was calculated according to Wilson (1927) to provide the 95% confidence intervals.

Results

For this study, 239 sequences of the ITS rDNA region were analyzed (21 of which, newly generated), 209 sequences of the *rpb2* gene (including 23 newly generated ones), 157 of the *ef1α* gene (37 of which were new), 140 of the IGS rDNA region (including 67 new ones) and 40 newly obtained sequences of *cox1* (Table 1S). The alignment lengths of the single locus alignments were 649 positions for ITS, 906 for *rpb2,* 619 for *ef1α,* 412 for IGS rDNA and 941 for *cox1*, respectively.

PCR success

The highest amplification success rate was obtained in amplifying *cox1* (83.5%), followed by IGS rDNA (72.2%) and ITS rDNA (60.4%) (Fig. 1). The amplification of *rpb2* was least successful, making it necessary in most cases to use nested-PCR to obtain sequences. Only when fresh material was used, we were able to obtain sequences without using a nested approach. Further, use of universal primers (Liu et al. 1999) had very little success, but also the use of more specific primers (Table 2S) did not yield in high success rates. The amplification success rate for the *ef1α* gene was similar to that of ITS rDNA region (59.6%); however, the former gene was amplified only using specific primers (Table 2S).

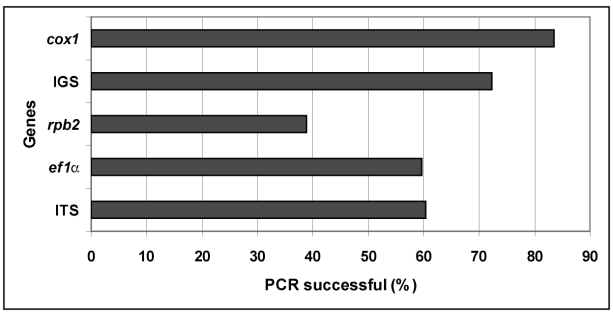

Fig. 1. PCR amplification success for each region.

Intraspecific and interspecific genetic divergence and barcoding gap

Figure 2 shows intra- and interspecific genetic distance distributions. In these histograms it can be seen that ITS rDNA, IGS rDNA, *rpb2*, and *ef1α* have somewhat overlapping intra- and interspecific distances. Table 1 summarizes the distance values for each locus and shows the quotient of inter-/ intraspecific distances. Only *cox1* has a barcoding gap, being 10 times the average intraspecific difference (Hebert et al. 2004). The other loci (ITS rDNA, IGS rDNA, *rpb2*, and *ef1α*) did not show barcoding gaps (Table 1). IGS rDNA showed the highest intra- and interspecific distance values. Wilcoxon signed-rank test showed that significant differences existed in the intraspecific variation among all the studied markers (Table 2). IGS rDNA showed the highest intraspecific divergence, and *ef1α* showed the lowest value. Wilcoxon signed-rank test also found significant differences in the interspecific variation (Table 3). The interspecific variations of ITS rDNA and IGS rDNA were similar, and larger than in the remaining markers. The interspecific divergence of *ef1α* was significantly lower than that of the remaining markers.

The species that showed the greatest intraspecific variation were: in the ITS rDNA region: *C. coniocraea, C. gracilis* and *C. humilis* (Table 4); in the IGS rDNA region: *C. ecmocyna, C. gracilis* and *C. macroceras;* in the *rpb2* region: *C. cariosa, C. ecmocyna,* and *C. gracilis;* in the *ef1α* region: *C. coniocraea, C. conista,* and *C. cornuta*, "barcoding gap".

Table 1 Ranges of intra- and interspecific genetic divergence for each locus, in brackets the average distance. The quotient between the average of inter- and intraspecific distances used to determine whether a barcoding gap exist, is also indicated.

Locus	Intraspecific	Interspecific	Inter-/Intraspecific
ITS rDNA	0.0-0.055742 (0.0094167)	0.00185-0.183526 (0.075871)	8.05
rpb2	0.0-0.070198 (0.00699763)	0.00-0.124629 (0.06579)	9.40
ef1α	0.0-0.0652 (0.0125)	0.0-0.10393 (0.04786)	3.83
IGS rDNA	0.0-0.21169 (0.0289)	0.00271-0.25309 (0.09063)	3.13
cox1	0.0-0.00428 (0.000697)	0.026161-0.04749736 (0.036624)	52.54

Table 2 Wilcoxon signed-rank tests of interspecific divergences among loci.

Comparison	Ranks	P-value	Results
IGS-*ef1α*	w+ = 3899331, w- = 16470	0.000*	IGS > *ef1α*
ITS-*ef1α*	w+ = 3891434, w- =24367	0.000*	ITS > *ef1α*
rpb2-*ef1α*	w+ = 38521587, w- = 63644	0.000*	*rpb2* > *ef1α*
ITS-IGS	w+ = 1210990, w- = 2704811	0.000*	IGS > ITS
rpb2-IGS	w+ = 686918, w- = 3228883	0.000*	IGS > *rpb2*
rpb2-ITS	w+ = 11176809.50, w- = 2738991.50	0.000*	ITS > *rpb2*

Table 3 Wilcoxon signed-rank tests of intraspecific divergences among loci.

Comparison	Ranks	P-value	Results
IGS-*ef1α*	w+ = 20220, w- = 3870	0.000*	IGS > *ef1α*
ITS-*ef1α*	w+ = 27639, w- = 4492	0.000*	ITS > *ef1α*
rpb2-*ef1α*	w+ = 18017, w- = 18981	0.000*	*rpb2* > *ef1α*
ITS-IGS	w+ = 18147, w- = 18981	0.748	IGS = ITS
rpb2-IGS	w+ = 29312, w- = 6734	0.000*	IGS > *rpb2*
rpb2-ITS	w+ = 4813, w- = 33137	0.000*	ITS > *rpb2*

Table 4 Intraspecific distances of the species studied (with 3 or more specimens) for each putative barcode.

Species	ITS rDNA	*rpb2*	IGS rDNA	*Cox1*	*ef1α*
C. acuminata	0.0-0.01960008 (0.01338574)	0.00-0.0070221 (0.0037509)	—	—	0.0-0.00326465 (0.00163159)
C. cariosa	0.0-0.036361 (0.02104244)	0.0-0.0384808 (0.0159689)	—	—	0.0-0.00986874 (0.00437325)
C. cervicornis	0.0-0.010582 (0.00492)	—	—	0.0-0.00119187 (0.0005835)	—
C. coniocraea	0.0-0.0370412 (0.019835)	0.0-0.0127123 (0.004344)	0.0-0.0729851 (0.02134)	—	0.0-0.06528902 (0.0230072)
C. conista	0.0-0.0076007 (0.002246)	0.0-0.0094787 (0.00396)	0.0-0.00598173 (0.001994)	—	0.0-0.0267257 (0.00926208)
C. cornuta	0.0-0.0258785 (0.006693)	0.0035378-0.0094705 (0.0061069)	0.0-0.02022391 (0.009763)	—	0.0-0.058865 (0.0328506)
C. corsicana	0.0-0.003527 (0.00176371)	0.0-0.001165 (0.0004659)	0.0-0.00544035 (0.002266)	—	—
C. cyathomorpha	0.0-0.0035211 (0.001182)	0.0-0.0029086 (0.00114166)	0.00587159-0.0117888 (0.007357)	—	0.0-0.0
C. ecmocyna	0.000	0.00842972-0.0226809 (0.015668)	0.0-0.124154 (0.0607492)	—	—
C. firma	0.0-0.0038557 (0.0019035)	0.0-0.0011338 (0.00046408)	—	0.0	—
C. foliacea	0.0-0.03555 (0.0078171)	0.0-0.032235 (0.012635)	—	0.0-0.00428043 (0.000721)	—
C. gracilis	0.0-0.046438 (0.020368)	0.00-0.070198 (0.019959)	0.0-0.211697 (0.046389)	—	—
C. hammeri	0.0-0.001657 (0.001657)	0.0-0.0065204 (0.0016856)	0.0-0.0269022 (0.01203)	—	0.0-0.0
C. humilis	0.0-0.055742 (0.008835)	0.0-0.00710009 (0.002354)	0.0-0.00882376 (0.0026315)	—	0.0-0.00163935 (0.00019314)
C. macroceras	—	—	0.0387099-0.135511 (0.084249)	—	—
C. nashii	0.003955-0.0181737 (0.0094705)	0.0-0.0028564 (0.0018066)	0.0-0.00958715 (0.0047997)	—	—
C. pulvinata	0.0-0.032891 (0.013029)	0.0-0.0022702 (0.0013236)	0.00853062-0.0355788 (0.023299)	0.0	—
C. rangiformis	0.0-0.0447728 (0.016282)	0.0-0.02625 (0.006176)	0.0-0.0197825 (0.006811)	—	—
C. rei	0.0-0.030073 (0.011906)	0.0-0.00982 (0.0036998)	—	—	0.0-0.0240494 (0.00772831)
C. subconistea	0.0-0.00883025 (0.0046704)	0.00117371-0.00825903(0.005501)	0.0-0.012603 (0.0083633)	—	0.0-0.0115919 (0.0056203)
C. subturgida	0.0-0.003552 (0.001236)	0.0-0.0065832 (0.0020228)	—	—	—
C. subulata	0.0-0.019336 (0.006859)	0.0-0.0130215 (0.002897)	0.0-0.0477285 (0.0231776)	—	0.0-0.0050157 (0.00196007)
C. symphycarpa	0.0-0.02953332 (0.01954)	0.0-0.0036345 (0.0017563)	—	—	0.0-0.00799591 (0.00263189)
Cladonia sp. 1	0.0-0.033409 (0.016089)	0.0-0.0117501 (0.005868)	—	—	0.0-0.0

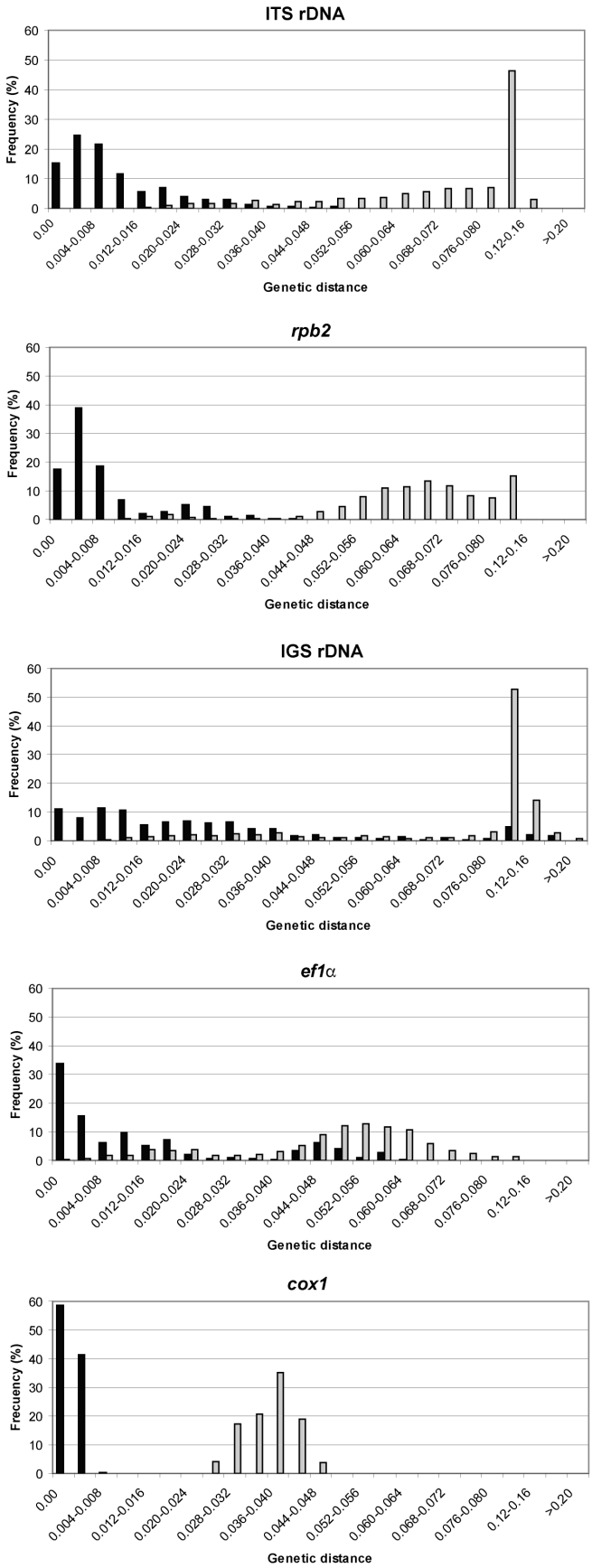

Fig. 2 Histograms with the frequency distribution of intra- and interspecific pairwise distances of ITS rDNA, *rpb2*, *ef1α*, IGS rDNA and *cox1*. The intraspecific distances are shown as grey bars and interspecific distances are shown as black bars.

PCI

PCI values varied from 0.78 to 1.00 for the different loci (Fig. 3). The marker that gives the highest PCI value was *rpb2*. ITS rDNA, IGS rDNA and *ef1α* gave similar values (0.78). The 2-locus combinations, which produced the highest PCI values were IGS rDNA-*rpb2* and ITS rDNA-*rpb2*. The 4-locus combination gave a PCI value of 0.89 (lower than that *rpb2*).

Discussion

The barcode for fungi, ITS rDNA (Schoch et al. 2012) has the unquestionable advantage that a large number of sequences are available in GenBank and that it is generally easily obtained. Nevertheless, in some species complexes of the genus *Cladonia,* ITS rDNA in not sufficient for discriminating among species (Fontaine et al. 2010; Kotelko & Piercey-Normore 2010; Pino-Bodas et al. 2011). In BLAST searches carried out by Kelly et al. (2011) using ITS rDNA for species identification, *Cladonia* was the genus that showed most failures. Thus we were examining other loci that, in combination with ITS, could improve species identifications of *Cladonia* species using DNA barcoding. The criteria to assess the suitability of a locus for barcoding are: i) it must be a short region (500-800 bp), ii) easily amplifiable, iii) with a low intraspecific variation, and iv) with higher interspecific than intraspecific variation (Letournear et al. 2010). Based on these criteria, we discuss in the following which of the loci examined would be best suited as an additional barcode for species identification in *Cladonia*. In general, the PCR success rates were lower than those found by other authors (Schoch et al. 2012; Zhao et al. 2011, 2012). This is probably due to the fact that a number of the material studied by us was not fresh material, but consisted largely of herbarium material.

None of the four loci most intensely studied here showed a barcoding gap (Table 1). The only region that showed a gap was *cox1*. This region has been used as barcode in animals (Hebert et al. 2003b, 2004) and in Oomycota (Robideau et al. 2011; Martín & Tooley 2003), but has not been widely used in fungi, especially because it has numerous introns (extant in 1 to 5% of samples) (Seifert et al. 2007; Nguyen & Seifert 2008; Feau et al. 2011) and it has too few conserved regions that permit designing universal primers (Seifert 2009). The studies which have used the *cox1* region in fungi have found inconsistent results. While some authors have reported that resolution to distinguish species in fungi was as good as that of ITS rDNA region or even better (Seifert et al. 2007; Nguyen & Seifert 2008), *cox1* was found insufficient for distinguishing species in other fungal groups (Geiser et al. 2007). Our results indicate that the average interspecific distance of *cox1* in *Cladonia* is more than 50x the intraspecific distance. On the basis of the widely used threshold for barcoding studies (10x; Herberg et al. 2004), and given the high success in the amplification of this region (83.5%), this region is promising as a second barcode for *Cladonia* spp. However, we have only limited data on *cox1* and hence additional studies on more *Cladonia* spp. are necessary to evaluate the performance of this locus. With the data at hand we cannot rule out that the discontinuity between intra- and interspecific distances might be an artifact resulting from the small sampling size.

The lack of a barcoding gap in ITS rDNA in *Cladonia* had been previously found by Kelly et al. (2011). Among genera analyzed in this study, only *Cladonia* and *Physcia* lacked a barcoding gap in ITS rDNA. Other barcoding studies on lichenized fungi also found a gap between intra- and interspecific distances, for

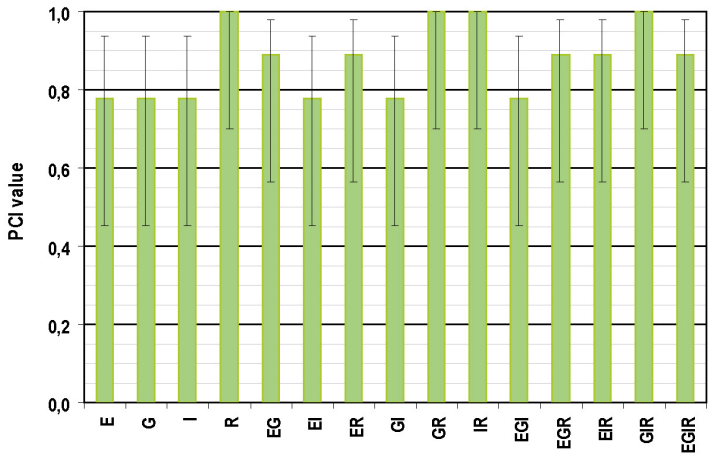

Fig. 3 The bars give the value of the monophyletic PCI for each locus and the different combinations of loci. E = *ef1α*, G = IGS rDNA, I = ITS rDNA, R = *rpb2*. The error bars indicate 95% confidence intervals, according to the Wilson score interval according to Wilson (1927).

example in Parmeliaceae, in which a threshold value of 0.017 has been established for the intraspecific distances (Del-Prado et al. 2010). The absence of a barcoding gap makes it impossible to provide a genetic distance threshold value for telling species apart in *Cladonia* for these markers. The lack of a barcoding gap has been found in numerous studies (Meier et al. 2006; Wiemers & Fiedler 2007; Shearer & Coffroth 2008; Divison et al. 2009; Langhoff et al. 2009; Hollingsworth et al. 2009). The authors explain this lack because of: incorrect taxonomy, hybrid species, incomplete lineage sorting or radiations, that is, recent lineages with a large number of species closely related (Wiemers & Fiedler 2007; Langhoff et al. 2009; Maia et al. 2012).

As for the power of ITS rDNA, IGS rDNA and *ef1α* for distinguishing species, it was found that the performance is similar (Fig. 3), but IGS rDNA showed the highest amplification rate. However, IGS rDNA is also the locus that shows the greatest intraspecific distance (Table 1, 3), potentially creating problems when using it as barcode in *Cladonia*.

The marker *ef1α* has been used as a barcode in different fungal groups, such as *Fusarium* (Geiser et al. 2004), *Tricholoma, Hypocrea* (Druzhinina et al. 2005), and Nectriaceae (Zhao et al. 2012). In some groups this marker was even more variable than ITS rDNA (Gazis et al. 2011). In *Cladonia* this marker had PCI values similar to those of ITS rDNA (Fig. 3) and showed the lowest intraspecific distances (Table 3). Besides, the amplification rate was almost as high as in ITS rDNA. However, the locus had the lowest interspecific variation (Table 2).

The *rpb2* locus gave the highest PCI value. In fact, this locus has been frequently used for phylogenetic studies in fungi (e.g., Reeb et al. 2004; Liu & Hall 2004; Buschbom & Mueller 2006; Hofstetter et al. 2007; Tehler & Irestedt 2007; Baloch et al. 2010). On the other hand, this locus also gave the lowest amplification success rate, which limits its use as barcode. In order to increase the amplification success rate, amplification of a shorter fragment should be tested (about 500-600 bp, instead of 950-1200 bp). We propose this region to be further studied as a possible barcode in *Cladonia*.

In barcoding studies, it is generally accepted that the combination of several loci increases the proportion of species distinguished (Kress et al. 2005; Fazekas et al. 2008; Edwards et al. 2008; Hollingsworth et al. 2009). Our results show, however,

that the combination of two or more genes does not always improve the species identification in *Cladonia* (Fig. 3). The combinations *ef1α*+*rpb2*, *ef1α*+*rpb2*+IGS rDNA, *ef1α*+ITS rDNA+*rpb2*, and likewise the four marker combinations, gave lower PCI values than those of some individual markers, while the combinations *ef1α*+ITS, ITS+IGS and *ef1α*+ITS+IGS did not improve PCI values. This agrees with other studies on DNA barcoding in which no improvement of the PCI values has been found in multilocus combinations (Pang et al. 2011). In most of the poorly performing combinations in our study *ef1α* is included, and we suspect that the low interspecific variation of this marker decreases the discriminating power of the remaining markers.

Intraspecific variation

Some authors use a 3%-threshold of sequence identity to identify conspecifity (Begerow et al. 2010). However, this value is not generally accepted in fungi, since it has been shown to be too high in some groups (Nilsson et al. 2008) and too low in others (Feibelman et al. 1994). In *Cladonia* we found that 94.2% of intraspecific comparisons of distances of ITS rDNA; 68.1% of IGS rDNA; 97.2% of *rpb2* and 83.5% of *ef1α* are lower than 3% (Fig. 2). The highest values of intraspecific distances have been found in those species for which sequences coming from a wide geographic range are available. Such is the case for *C. coniocraea* (Table 4), which showed a high intraspecific variation ITS rDNA and in *ef1α*, markers for which sequences were obtained from samples coming from Europe and North and South America (Table 1S). The remaining species with samples coming from distant geographic areas and with high values of intraspecific genetic distances include *C. cariosa*, *C. conista,* and *C. humilis,* whose samples came from Europa and North America. This confirms that it is important to include samples from the entire geographical range of a species when assessing intraspecific variation.

One of the species showing the highest genetic divergence for all the markers in our study was *Cladonia gracilis*. This indicates that the taxonomy of the species requires further studies. In fact, a number of subspecies have been described for this species (Ahti 1980), indicating that additional species might be hidden under the name. However, molecular studies carried out so far have not been able to clarify the taxonomy of this species complex (Fontaine et al. 2010; Pino-Bodas et al. 2011).

Conclusion

Our results indicate that *cox1* and *rpb2* are potential candidates as a second barcode marker in *Cladonia* in addition to ITS rDNA. But, further studies should be done to decide which of them is the best suited.

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DISCUSIÓN

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En el estudio taxonómico de cualquier grupo de organismos, distinguir entre los caracteres que están influenciados por la condiciones ambientales y aquellos que están relacionados con la variabilidad genética del propio organismo es clave para la delimitación de las especies. Con los estudios realizados en esta tesis doctoral se ha pretendido establecer los caracteres morfológicos y químicos con valor taxonómico en varios complejos de especies del género *Cladonia*. Para delimitar las especies se ha seguido el criterio de especies filogenéticas mediante concordancia genealógica (Taylor et al. 2000), que es el más extendido en hongos liquenizados. Nuestros análisis han demostrado que, según dicho criterio, muchas de las especies de *Cladonia* son inconsistentes. Sobre la base de nuestros resultados y los publicados por otros autores, hemos evaluado y reinterpretado los principales caracteres morfológicos, anatómicos y químicos utilizados hasta ahora para distinguir algunas de las especies del género *Cladonia.*

CARACTERES MORFOLÓGICOS

Así, el **tamaño de las escuámulas del talo primario** es un carácter que se ha sobrevalorado en muchas ocasiones. Este carácter se utilizó sobre todo para distinguir especies en la sección *Helopodium* (Stenroos 1988), la cual contenía muchas especies que sólo en raras ocasiones desarrollan podecios, por lo que era necesario utilizar los caracteres asociados al talo primario para distinguir las especies. Especies de este grupo se han analizado en los ARTÍCULOS I, II y IV. Los resultados de los ARTÍCULOS I y II demuestran que las diferencias en el tamaño del talo primario no se correlacionan con la variación genética, sino que constituyen una respuesta a las diferentes condiciones ambientales, tales como el tipo de sustrato y el grado de aridez. Kotelko & Piercey-Normore (2010) demostraron que las diferencias morfológicas del talo primario utilizadas para distinguir *C. pyxidata* de *C. pocillum* (escuámulas erectas o paralelas al sustrato, separadas o imbricadas, con superficie entera o cuarteada) están directamente relacionadas con el pH del suelo sobre el que se desarrollan los talos. Es muy probable que factores como la humedad y los contrastes de temperatura influyan en el tamaño de las escuámulas. Sin embargo, en el grupo de *C. cariosa* (ARTÍCULO IV) se encontró que existen diferencias significativas en el tamaño de las escuámulas entre los clados A y B y entre los clados A y D, por lo que el tamaño de las escuámulas podría ser utilizado, en combinación con los caracteres de los podecios y el córtex, para distinguir las especies de este grupo.

Tanto el **tamaño de los soredios** como **su localización** han sido caracteres taxonómicos muy utilizados en *Cladonia.* Tal y como se detalla en el ARTÍCULO III, el tamaño de los soredios permite distinguir especies estrechamente relacionadas en numerosos grupos, como en el complejo *C. chlorophaea*-*C. fimbriata* (Ahti 1966; Hennings 1983). En los complejos de especies estudiados, el tamaño de los soredios fue uno de los caracteres utilizados para distinguir *C. subulata* de *C. rei* y *C. coniocraea* de *C. ochrochlora*, así como las especies del complejo de *C. humilis*. Mientras que en *C. subulata* y *C. rei* las diferencias en el tamaño de los soredios pueden ser utilizadas como un carácter adicional para la distinción de especies (ARTÍCULO III), en el complejo de *C. humilis* las morfoespecies con diferente tamaño de soredios no siempre constituyen grupos monofiléticos (ARTÍCULO VII). Por ejemplo, *C. kurokawae* (con gránulos) y *C. subconistea* (con soredios farináceos) han

resultado ser coespecíficas. Tampoco las especies con un tamaño de soredios similar están estrechamente relacionadas (ARTÍCULO VII). Este es el caso de *C. conista*, *C. humilis* y *C. nashii,* todas ellas con soredios farináceos pero que no están estrechamente relacionadas. Por otro lado, *Cladonia coniocraea* y *C. ochrochlora,* a las que se les había atribuido, entre otras diferencias morfológicas, diferente tamaño de soredios, tampoco constituyen linajes independientes (ARTÍCULO V). También, de acuerdo con Stenroos et al. (2002) la presencia de podecios sorediados es un carácter homoplásico en el género; incluso dentro de pequeños grupos de especies como el subgrupo *Graciles,* los podecios sorediados han aparecido más de una vez a lo largo de la evolución del grupo (ARTÍCULO V). Sin embargo, este carácter puede ser utilizado para distinguir especies en dicho grupo, puesto que los taxones del subgrupo *Graciles* que desarrollan podecios sorediados (*C. coniocraea* y *C. cornuta*) lo hacen siempre. Hay que destacar, sin embargo, que la independencia de *C. cornuta* no ha sido resuelta.

Los **podecios escifosos** han aparecido numerosas veces a lo largo de la evolución del género *Cladonia* (Stenroos et al. 2002). En aquellas especies que llevan soredios o placas que actúan como propágulos vegetativos, los escifos se han interpretado como estructuras de gran importancia para la dispersión de dichos propágulos vegetativos (Bailey 1966). El diámetro de los escifos fue uno de los caracteres utilizados para distinguir las diferentes subespecies de *C. gracilis* (ARTÍCULO V), subespecies que, sin embargo, no constituyeron linajes monofiléticos independientes. De hecho, este carácter ha resultado tener un grado de homoplasia alto (ARTÍCULO V), por lo que no debería usarse para distinguir especies. Fontaine et al. (2010) sugieren que en el grupo de *C. gracilis* los escifos pueden tener la función de almacenar agua cerca de la zona donde se desarrollan los apotecios, ya que para que estos se desarrollen es posible que se necesite una cierta cantidad de humedad. Esta hipótesis podría explicar la inconstancia de los escifos en la mayor parte de los taxones del grupo de *C. gracilis*, cuya presencia estaría vinculada a estados de desarrollo del talo que producen apotecios. Hammer (1998) encontró en *C. subcervicornis* que los mismos procesos morfogenéticos que producen escifos son los que dan lugar a las ramificaciones de los podecios. Por tanto, desde el punto de vista ontogénico, en *C. subcervicornis* no tiene sentido distinguir entre escifos y ramas. El autor considera probable que la disponibilidad de luz influya sobre los procesos de morfogénesis del talo. En este caso, la presencia de escifos no es un carácter útil para distinguir especies.

El **tipo de ramificación de los podecios** ha sido uno de los caracteres fundamentales para la identificación de especies en *Cladonia* (Ahti 2000). Sin embargo, en algunos casos las diferencias en el tipo de ramificación han sido mal interpretadas. En *C. arbuscula* se describieron varios taxones infraspecíficos con diferencias en el número de ramificaciones: *C. arbuscula* subsp. *squarrosa,* con podecios densamente ramificados, y *C. arbuscula* subsp. *beringiana,* con podecios menos ramificados (Ruoss 1987), pero estas diferencias morfológicas no están correlacionadas con diferencias genéticas (Piercey-Normore et al. 2010). En otros casos, el tipo de ramificaciones puede variar durante las fases de desarrollo de la especie, como ocurre en *C. subulata* y *C. rei* (ARTÍCULO III), lo que dificulta el uso de este carácter para distinguir especies estrechamente relacionadas. La presencia de podecios poco ramificados permite discriminar *C. symphycarpa* s.s. (clado B) de los linajes A y D del grupo de *C. cariosa* (ARTÍCULO IV). En el grupo de *C. furcata* el tipo de ramificaciones y la presencia de axilas abiertas o cerradas (algunos de los caracteres utilizados para distinguir a *C. furcata* de *C. subrangiformis*) no reflejan

ningún patrón filogenético (ARTÍCULO VIII), sino que en ambos clados aparecen especimenes provistos de ramas espinosas o sin ellas, ramificaciones con ángulo agudo u obtuso y axilas abiertas o cerradas.

Los caracteres asociados al **córtex de los podecios** han sido estudiados en varios complejos de especies. En el ARTÍCULO III se demuestra que la presencia o ausencia de córtex en la base de los podecios no es un carácter discriminante para distinguir *C. subulata* de *C. rei*. En el ARTÍCULO IV las diferencias en el córtex, continuo o fisurado, permiten distinguir el clado B (formado por *C. symphycarpa*) del resto. Sin embargo, los especímenes de los clados A y D tienen podecios con córtex fisurado. En el ARTÍCULO VII, especímenes con similares características del córtex aparecen en diferentes clados. Del mismo modo, varios clados agrupan a especímenes cuyo córtex es diferente. En resumen, las características del córtex pueden ser utilizadas, en algunos casos, como caracteres discriminantes entre especies afines, pero en otros no.

Los **caracteres anatómicos** están ganando importancia para diferenciar especies estrechamente relacionadas en los hongos liquenizados. En Parmeliaceae, los estudios filogenéticos revelaron que los géneros y especies se dividían en varios clados monofiléticos (Blanco et al. 2004; Argüello et al. 2007; Divakar et al. 2010). Las revisiones posteriores de los especímenes de cada uno de los clados encontraron, en muchos casos, diferencias anatómicas que habían pasado inadvertidas. En otras familias, como Physciaceae (Divakar et al. 2007), Pertusariaceae (Schmitt & Lumbsch 2004) o Verrucariaceae (Prieto et al. 2012), caracteres anatómicos tales como el tipo de córtex, el tipo de medula o la estructura de la pared de las ascósporas han resultado ser útiles para distinguir especies afines o grupos de especies con una historia evolutiva común. En las Cladoniaceae, los géneros *Heterodea* (con talos foliáceos) y *Ramalinora* (con talo crustáceo) constituyen un grupo monofilético con *Cladia* (con pseudopodecios) (Parnmen et al. 2010; Lumbsch et al. 2010). Los estudios anatómicos revelaron semejanzas en la anatomía entre el talo foliáceo de *Heterodea*, el crustáceo de *Ramalinora* y los pseudopodecios de *Cladia,* indicando que son estructuras homólogas. En la presente memoria doctoral se han realizado estudios anatómicos en varios de los grupos de estudio. En el ARTÍCULO III se encontró que la superficie del estereoma de *C. subulata* es reticulada mientras que la de *C. rei* es lisa. Sin embargo, la necesidad de usar microscopía electrónica de barrido para observar dichas diferencias limita este carácter en las identificaciones rutinarias. Caracteres asociados al estereoma se habían utilizado con anterioridad para discriminar especies estrechamente relacionadas en *Cladonia* (Ahti 2000). Uno de los caracteres de *C. furcata* es la presencia de un esteroma estriado; sin embargo, en el ARTÍCULO VII no se ha encontrado que este carácter esté asociado con ninguno de los dos grupos monofiléticos obtenidos. En el ARTÍCULO IV se encontró que la estructura del córtex del talo primario permitía diferenciar especies en el grupo de *C. cariosa*, ya que la estructura del córtex de los especímenes de cada uno de los clados era diferente Así, el córtex de los especímenes del clado A contiene una capa epinecral no presente en el resto de clados. La superfice del córtex es diferente en los especímenes de los diferentes clados, desde lisa, con fisuras anchas y poco profundas, areolada-verrucosa, hasta fuertemente fisurada. Los resultados obtenidos durante el desarrollo de esta tesis, junto con las evidencias encontradas con anterioridad por otros autores, ponen de manifiesto la gran importancia de los caracteres anatómicos para diferenciar especies.

CARACTERES QUÍMICOS

Los **metabolitos secundarios o extrolitos**, muy utilizados para la delimitación de especies en los hongos liquenizados, están perdiendo importancia en algunos grupos debido a que no predicen linajes filogenéticos (Velmala et al. 2009; Leavitt et al. 2011; Myllys et al. 2011). En *Cladonia*, los primeros estudios de secuencias de ADN mostraron que los diferentes quimiótipos en el grupo de *C. chlorophaea* (habitualmente reconocidos como especies diferentes) no se diferenciaban genéticamente (DePriest 1994). Este resultado fue confirmado por Dolnik et al. (2010) para *C. novochlorophaea* (contiene ácido homosekikaico) y *C. merochlorophea* (contiene ácido merocloroféico y ácido fumarprotocetrárico) y por Beiggi & Piercey-Normore (2007) en *C. chlorophaea*, *C. grayi* y *C. merochlorophea*. Los resultados de los ARTÍCULOS II, III, IV, VII, VIII muestran que existen varios clados monofiléticos que poseen varios quimiótipos (*C. subturgida, C. rei, C. symphycarpa, C. cariosa, C. acuminata, C. hammeri, C. nashii, C. subconistea, C. subrangiformis*), algunos de ellos considerados con anterioridad como especies diferentes. Otros autores han encontrado resultados semejantes en otros complejos de especies en *Cladonia* (Lendemer & Hodkinson 2009; Piercey-Normone et al. 2010). Estos resultados verifican la primera parte de la segunda hipótesis planteada en esta memoria doctoral: la producción de diferentes metabolitos secundarios no siempre refleja la existencia de diferentes linajes evolutivos.

Varios autores postularon que la presencia o ausencia de metabolitos secundarios producidos por diferentes rutas metabólicas podría indicar mayor diferencia genética que la presencia de dos metabolitos secundarios sintetizados por la misma ruta. Este último caso podría indicar la existencia de una mutación en un solo gen (Rogers 1989). En los ARTÍCULOS II, IV, VII y VIII encontramos clados cada uno de los cuales incluye quimiótipos cuyos metabolitos secundarios son sintetizados por diferentes rutas metabólicas. De acuerdo con el ARTÍCULO II, *C. subturgida* puede contener ácido fumarprotocetrárico y atranorina (una depsidona y un dépsido respectivamente, ambos de la serie del ß-orcinol), u otros quimiótipos que contienen ácido protoliquesterínico, un ácido graso que, aunque se sintetiza por la ruta del acetato-malonato, es una ruta independiente de la serie del ß-orcinol. En el ARTÍCULO III encontramos que *C. cariosa* s.s. puede contener quimiótipos cuyos compuestos son sintetizados por la serie del ß-orcinol, como el ácido fumarprotocetrárico y la atranorina, junto con otros que contienen ácido rangifórmico, un ácido graso. En los ARTÍCULOS VII y VIII de nuevo nos encontramos con que varios clados (el formado por *C. humilis, C. hammeri*, y el clado A del grupo de *C. furcata*) contienen metabolitos de la serie del ß-orcinol y otros que contienen el ácido bourgeánico, un ácido graso. Además, existen algunos quimiótipos en los que aparecen juntos compuestos sintetizados por distintas rutas metabólicas. Este es el caso del quimiótipo de *C. rei* (ARTÍCULO III) que contiene ácido homosekikaico, un dépsido de la serie del orcinol, junto con ácido fumarprotocetrárico, una depsidona de la serie del ß-orcinol. En los ARTÍCULOS II, IV, VII y VIII también encontramos quimiótipos en los que aparecen juntos metabolitos de diferentes rutas metabólicas, como la zeorina (un triterpeno, sintetizado por la ruta del ácido mevalónico), la atranorina (dépsido de la serie del ß-orcinol), el ácido fumarprotocetrárico (depsidona de la serie del ß-orcinol), el ácido rangifórmico o el ácido bourgeánico (ácidos grasos). En otras especies de *Cladonia* (*C. secundana*, *C. peltasica* y *C. squamosula),* el ácido homosekikaico (dépsido de la

serie del orcinol) y la atranorina aparecen juntos (Huovinen & Ahti 1986; Huovinen et al. 1989). Estos hallazgos sugieren que la presencia de metabolitos sintetizados por diferentes rutas metabólicas no predice la existencia de diferencias genéticas discriminadoras de especies entre dichos quimiótipos. Sin embargo, en el ARTÍCULO VI se encontró que el carácter más fiable para distinguir *C. humilis* de *C. conista* es la presencia o ausencia de ácido bourgeánico.

Sobre la base de nuestros resultados, apoyamos la postura de Lumbsch (1998), ya que a priori no se puede asegurar que la variación química tenga valor en la delimitación de especies, siendo necesario el estudio particular de cada grupo para determinar el valor taxonómico de los metabolitos secundarios.

DIVERSIFICACIÓN EN *CLADONIA*

Aún estamos lejos de conocer cuándo se originó y cuáles fueron los procesos evolutivos que condujeron a la diversificación del género *Cladonia*. Serán necesarios estudios de datación que incluyan un número representativo de taxones de este género, así como realizar análisis de reconstrucción de áreas para conocer el centro de diversificación del género. Los datos que se tienen hasta la fecha indican algunos de los procesos que pueden haber contribuido a la diversificación en especies. En algunos casos hay evidencias de divergencia simpátrica probablemente originada por especiación ecológica (Schluter 2000, 2001; Rundle & Nosil 2005); es decir, el proceso por el cual la selección ecológica divergente provoca que se establezcan barreras en el flujo génico entre las poblaciones. Este es el caso del grupo de *C. cariosa* (ARTÍCULO III), así como de *C. humilis* y *C. conista* (ARTÍCULO VI). En el grupo de *C. cariosa* los especímenes del clado D se encontraron en localidades cuyas condiciones ecológicas son diferentes a las del resto de los clados (suelo ácido y mayor altitud), por lo que este tipo de especiación podría haber tenido lugar.

En el género *Cladonia* existen numerosas especies con amplia distribución. En otros grupos de líquenes se ha encontrado que las especies de amplia distribución (incluyendo varios continentes) escondían especies crípticas (Crespo & Pérez-Ortega 2009). Este hallazgo podría extenderse a las especies de *Cladonia*, pero no se ha encontrado una relación entre los linajes filogenéticos y el origen geográfico (ARTÍCULOS III, IV, VI, VII). Myllys et al. (2003) encontraron que existía un flujo génico entre las poblaciones del hemisferio Norte y Sur de *C. arbuscula.* La ausencia de divergencia genética entre las poblaciones alejadas de especies con amplia distribución puede ser debida a que la mayoría de especies de este género solo en raras ocasiones se reproduce de forma sexual. De modo que una combinación entre la dispersión a larga distancia de los propágulos vegetativos (Muñoz et al. 2004) y la ausencia de reproducción sexual explicaría la falta de diferenciación entre poblaciones muy alejadas. Especies crípticas han sido encontradas en el grupo de *C. pyxidata* (Kotelko & Piercey-Normore 2010). Sin embargo, los distintos linajes filogenéticos no tienen diferente distribución.

Hipótesis sobre otros procesos de especiación, como la hibridación, incluida la hibridación somática (fusión de hifas entre individuos genéticamente diferentes), se han planteado para explicar los patrones filogenéticos encontrados en *Cladonia* (Kotelko & Piercey-Normore 2010). La hibridación somática, también conocida como hibridación mecánica (Hawksworth 1978) fue sugerida en las Cladoniaceas como una interpretación a la existencia de especímenes con morfologías aberrantes o con combinaciones de extrolitos poco frecuentes. Se propuso que los propágulos de diferentes especies que crecían juntas podían formar un único talo con hifas de

ambos parentales (Jahns 1972; Hawksworth & Hill 1984; Hawksworth 2000). Aunque esta hipótesis aún no ha sido demostrada, se han encontrado ciertas evidencias de hibridación somática en *C. arbuscula*. Robertson & Piercey-Normore (2007) encontraron diferentes copias de la región ITS rDNA en una única muestra proponiendo, entre otras posibles explicaciones, la posibilidad de que el podecio estuviese formado por dos individuos.

En géneros como *Cladonia*, donde la identificación de especies requiere un gran conocimiento sobre la variabilidad morfológica de los taxones, la aplicación de la técnica del código de barras genético (DNA barcoding, (Herbert et al. 2003) puede ser muy útil. En el ARTÍCULO IX se llevó a cabo un estudio para evaluar cuál de las regiones génicas estudiadas en los artículos precedentes era más eficaz para distinguir las especies. Los resultados de este estudio indicaron que, además de la región ITS rDNA, *cox1* y *rpb2* pueden ser potenciales códigos de barras (barcodes) en *Cladonia*. El mayor inconveniente del gen *rpb2* es la dificultad de amplificarlo. Sin embargo, su capacidad para resolver especies es muy alta. El número de especies estudiadas aquí es limitado en comparación con el número total de especies del género y para evaluar la capacidad de los diferentes loci como códigos de barras (barcodes) es importante incluir el máximo número de especies. Por tanto, los resultados obtenidos en el ARTÍCULO IX deberán ser confirmados con un muestreo más amplio de especies.

PERSPECTIVAS FUTURAS

En esta tesis doctoral se ha abordado la delimitación de especies en numerosos grupos de especies afines con amplia representación en la región Mediterránea. Sin embargo, en el género *Cladonia* quedan muchos otros complejos en los cuales los límites entre las especies no están claros. Tanto los autores del ARTÍCULO I como Beiggi & Piercey-Normore (2007) encuentran que el grupo de *C. verticillata* (que comprende alrededor de 20 especies en todo el mundo) necesita una revisión a nivel taxonómico. Ahti (2000) declara la necesidad de un mayor estudio para la delimitación de *C. cartilaginea* Müller Argaviensis, que es muy variable y cuyos quimíotipos necesita ser revisado. Esta se confunde morfológicamente con *C. corymbites* Nyl. Además, algunos especimenes de *C. corymbites* de Sudamérica pueden ser un taxon diferente. El trabajo filogenético de Stenroos et al. (2002) pone de manifiesto la necesidad de revisar el complejo de *C. uncialis* (L.) F. H. Wigg., especie circumpolar distribuida desde las zonas árticas a las templadas del hemisferio Norte, que contiene dos subespecies, *C. uncilais* subsp. *uncialis* y *C. uncialis* subsp. *biuncialis* (Hoffm.) M. Choisy. En este trabajo, algunos de los grupos de estudio no han sido completados, por ejemplo, será necesario trabajo adicional para aclarar si *C. farinacea* de Norteamérica y de Chile son coespecíficas o son especies independientes. La identidad de *C. furcata* y *C. subrangiformis* no ha sido resuelta, y el estudio de más loci es necesario para resolver dicho complejo. El grupo de *C. cariosa* necesita más estudio, incluyendo todos los quimiótipos descritos. El grupo de *C. pyxidata* fue tratado por Kotelko & Piercey-Normore (2010), sin embargo el número de especies contenidas en el grupo no se ha determinado, siendo necesario un estudio más exhaustivo que incluya un gran número de especímenes de las especies europeas *C. monomorpha* y *C. magyarica*. Cuando se aborde el estudio de estos grupos será necesario utilizar los nuevos métodos filogenéticos de coalescencia, con objeto de estudiar la separación incompleta de linajes.

Las categorías infragenéricas propuestas por Stenroos et al. (2002) son todavía provisionales, a la espera de que puedan establecerse nuevas secciones representadas por clados monofiléticos. Stenroos et al. (2002) exploraron alrededor del 35% de las especies descritas, por lo que un muestreo de taxones más amplio (cercano al 90%), así como utilizar múltiples loci será necesario para establecer una nueva clasificación infragenérica.

CONCLUSIONES

CONCLUSIONES

De acuerdo con los objetivos planteados y los resultados obtenidos sobre la delimitación de algunas especies conflictivas en el género *Cladonia* (que tiene amplia representación en la región Mediterránea), de esta memoria doctoral se concluye:

1- Sobre la base del reconocimiento filogenético de especies mediante concordancia de genealogías, muchos de los taxones de *Cladonia* analizados han resultado ser coespecíficos (*Cladonia convoluta*/*C. foliacea; C. iberica*/*C. subturgida*; *C. coniocraea/ C. ochrochlora* y *C. cornuta* subsp. *groenlandica; C. kurokawae/C. subconistea*). La variación de los caracteres morfológicos habría llevado a sobreestimar el número de especies en este género. En general, los caracteres cuantitativos, tales como el tamaño de las escuámulas, la anchura de los escifos y el tamaño de los soredios tienen un limitado valor taxonómico para distinguir especies estrechamente relacionadas.

2- El tamaño de las escuámulas del talo primario está muy afectado por las condiciones ambientales y, en general, no debe usarse como carácter discriminante para la identificación de especies. Sólo en combinación con otros caracteres puede ser útil en las identificaciones.

3- La forma y el tamaño de los propágulos vegetativos solo puede ser utilizado como carácter adicional para la distinción de especies ya que es variable dentro de los taxones.

4- La anchura de los escifos no debe usarse como carácter taxonómico para distinguir especies, ya que presenta una gran plasticidad fenotípica.

5- Los caracteres anatómicos, tales como la estructura del córtex y el estereoma, deben ser mejor explorados, puesto que han resultado ser útiles para la discriminación de especies de *Cladonia*.

6- La presencia de metabolitos secundarios de un tipo u otros no implica la existencia de linajes independientes. De hecho, existen numerosos linajes químicamente variables (p. ej., *C. acuminata*, *C. cariosa*, *C. furcata*/*C. subrangiformis*, *C. hammeri*, *C. nashii*, *C. subturgida*, *C. subconistea, C. symphycarpa*). La presencia de metabolitos secundarios sintetizados por diferentes rutas metabólicas, puede ser a veces caracter diagnóstico (p. ej., *C. conista* y *C. humilis*), pero no en todos los casos (p.ej., no es un carácter diagnóstico para distinguir *C. pulvinella* de *C. hammeri*, o *C. subturgida* de *C. iberica*).

7- La ausencia de monofilia en muchos de los taxones estudiados (p.ej., los taxones del grupo de *C. gracilis*; la especie *C. pulvinella* y las poblaciones europeas de *C. hammeri*) es probable que sea debida a la divergencia reciente de las especies.

8- El uso de regiones para código de barras (barcode) permitirá en un futuro identificar de forma correcta las especies de *Cladonia.* Sin embargo, deben

explorarse nuevas regiones, ya que, aunque *rpb2* es la región que presenta un mayor número de identificaciones correctas, es muy difícil de amplificar, lo que limita su utilidad como secuencia para código de barras (barcode).

CONSIDERACIÓN FINAL

La diversidad del género *Cladonia* está aún lejos de ser bien conocida. Los resultados de este trabajo ponen de manifiesto que emplear el reconocimiento filogenético de especies mediante concordancia de genealogías es importante en la sistemática del género; sin embargo, no es un sustituto de los datos morfológicos, anatómicos y químicos. La integración de todos los datos será lo que en un futuro nos permita avanzar en el entendimiento de la biodiversidad de *Cladonia*.

CONCLUSIONS

In relation to the aims addressed in this doctoral thesis, and according to the results obtained in it about the delimitation of some controversial species within the genus *Cladonia* (which has a broad representation in the Mediterranean region), the following can be concluded:

1- On the base of genealogical concordance phylogenetic species recognition, many of the analyzed taxa within the genus *Cladonia* have turned out to be conspecific (*Cladonia convoluta*/*C. foliacea; C. iberica*/*C. subturgida*; *C. coniocraea*/*C. ochrochlora* and *C. cornuta* subsp. *groenlandica; C. kurokawae*/*C. subconistea*). The variation of morphological characters would have led to overestimate the number of species within the genus. In general, quantitative characters such as the squamules size, the scyphi wideness and the soredia size have a limited taxonomical value in order to distinguish closely related species.

2- The primary thallus squamules size is very affected by environmental conditions and, in general, should not be used as a discriminating character for species identification. Only in combination with other characters it could be useful for identifications.

3- The form and size of the vegetative propagules only as additional characters can be used for species distinction, since they are variable within the different taxa.

4- The scyphi broadness should not be used as a taxonomical character for distinguishing species, as it shows a high phenotypical plasticity.

5- Anatomical characters, such as the cortex or the stereome structure ought to be further researched, since they have proved to be useful for species differentiation within *Cladonia*.

6- The presence of diverse secondary metabolites does not imply the existence of independent lineages. In fact, a considerable number of chemically variable lineages exist (e.g. *C. acuminata*, *C. cariosa*, *C. furcata*/*C. subrangiformis*, *C. hammeri*, *C. nashii*, *C. subturgida*, *C. subconistea, C. symphycarpa*). The presence of secondary metabolites synthesized through different metabolic pathway can be, in some cases, a diagnostic character (e.g. *C. conista* and *C. humilis*). But it is not so in all cases (e.g. it is not a diagnostic character to distinguish *C. pulvinella* from *C. hammeri*, or *C. subturgida* from *C. iberica*).

7- The absence of monophyly in many of the studied taxa (e.g. the taxa in *C. gracilis* group; the species *C. pulvinella*; and the European populations of *C. hammeri*) is probably due to the recent divergence of the species.

8- The use of genetic regions as barcodes will permit in a future to correctly identify the species within *Cladonia.* However, new genitic regions must be explored, since *rpb2* (the region wich produces most right identifications) is very difficult to amplify, what limits its usefulness as barcode.

FINAL CONSIDERATION

The diversity of the genus *Cladonia* is still far from being well known. The results of the present work make it clear that using genealogical concordance phylogenetic species recognition is important in the systematics of the genus; this method, however, is not a substitute for morphological, anatomical and chemical data. It is the integration of all the available data what, in a future, will permit us to advance in the understanding of the genus *Cladonia* diversity.

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ANEXOS

ANEXO 1: Material estudiado

***Cladonia acuminata* (Ach.) Norrl.**

Canada: Manitoba: Near S shore of Cedar Lake, km 4.1 on Mossy Portage Road, 25-V-2004, *T. Ahti 66130*, H; **Chile**: Región XII, Magallanes y Antártica Chilena: Puerto Natales, hacia P. N. Torres del Paine, laguna Amarga, 29-I-2005, *A. R. Burgaz*, MACB 92017; **Estonia**: Pärnu: Lodja, 30 Km SSE of Pärnu, 9-X-1977, *T. Ahti*, H; Harju: Tallin, Liiva Krug, Auf der Erde, 18-XI-1931, *J. Ruubel*, H; **India**: Jammu and Kashumir: Srinagar, Gulmarg, 12-IX-1982, *A. Singh & D. K. Upreti*, H; **Italy**: Trentino: Gesellig mit der Stammform auf begrastem Boden längs einer Waldlichtung bei Paneveggio, Südtirol, 1-VIII-1884, *Arnold*, H; **Norway**: Nord-Troms: Tromsö amt, Strömsör, 8-VI-1911, *B. Lynge*, H; Nord-Norge: Finnmark, Nesseby, Reppen, E of Repparfjordelva, 22-VIII-1974, *T. Ahti*, H; **Russia**: Perm: Cherdin, air station, 18-VII-2005, *L. Gagarina*, H; Republic Tuva: South Siberia Mountains, Todginskaya Valley, 8-VIII-1999, *T. N. Otnyukova*, H; Todginskaya Valley, Toora-Khem River in its low stream (Biy-Khem River bassin), Larch forest in the Toora-Khem River valley, 2-VII-1999, *T. N. Otnyukova*, H; Krasnoyarsk: North of Central Siberia, Taimyr Peninsula, near Khatanga settlement by Khatanga River, 4-IX-1995, *M. Zhurbenko*, H; North of Central Siberia, Taimyr Peninsula, near Khatanga settlement by Khatanga River, 4-IX-1995, *M. Zhurbenko*, H; **Spain**: Palencia: Puerto de Piedrasluengas, 29-IX-1999, *A. R. Burgaz*, MACB 92739; **Sweden**: Jämtland: Enafors, N of Enaforsholm, 11-VIII-1975, *T. Ahti*, H; Lappland: Lule, Lappmark, Jokkmokk, Muddus, National Park, 10 Km N of Messaure, Maskokarso Canyon, 15-VIII-1959, *T. Ahti & E. Uggla*, H; Asele Lappmark, Vihelmina, 20 Km ESE of Saxnäs, river Kultsjöan, by rapids Dimforsen, near bridge, 6-VIII-1991, *T. Ahti*, H; Asele Lappmark, Vihelmina, 10 Km E of Klimpfjäll, hill Röberget by lake Kultsjön, S slope, 7-VIII-1991, *T. Ahti*, H; Asele Lappmark, Risbäck, Mt. Kalvberget (0.5 Km WNW of Risbäck), 8-VIII-991, *T. Ahti*, H; Central Highlands, Arnarfellsver, SE of Hofsjökull Glaciar, 15-VIII-1972, *H. Kristinsson*, H; Lule Lappmark, Jokkmokk, 12 Km NW of Messaure, bank river Stora Luleälv, 16-VIII-1959, *T. Ahti & F. Skuncke*, H; **United States**: Alaska: North Slpoe Co., Noatak National Preserve, western Brooks Range, De Long Mountains, watershed of Kukururok River, 25-VI-2004, *T. Ahti 63278*, H.

***Cladonia apodocarpa* Robins**

United States: Pennsylvania: Huntingdon county, Told Towship, Trough Creek state Park, picnic area along Great Trought Freek 1 mi N of park office, 22-IV-208, *L. C. Harris 54250*, H.

***Cladonia callosa* Harmond**

United Kingdom: Scotland: Agyllshire, head of Loch Craigneish, by side of the A816 where the Allt Ath Mhic Mhartein runs under the road, 24-VI-1976, *P. W. James*, H; **Germany**: Schlewing-Heide, 13-IX-1992, *S. Paus*, H.

***Cladonia cariosa* (Ach.) Sprengel**

Austria: Styria: Steirisches Randgebirge, Stubalpe, way from Weiβkirchen to the Hirscheggen Sttel, Kothgraben SE of Kleinfeistritz, near the Samerhütte, 27-V-1994, *J. Hafellner & H. Köckinger*, H; **Canada**: Alberta: Bow River Watershed, soth of Waiparous Creek, 9 miles west of the Cochrane, Nordegg Road, 19-VIII-1965, *C. D. Bird & J. L. Glenn*, H; British Columbia: Haines Triangle, near B. C. Yukon Border, Survey Lake, near headwater of Tatshenshini, 25-VII-1992, *T. Goward, D. Meldinger, R. Tranbridge & B. Callan*, H; Mountain side, south of Valemount, along Route 5, 25-VII-1985, *J.I. Crame & J. D. Schoknecht*, H; Shuswap Highland, Wells Gray Provincial Park, Ray's Farm, 3-VIII-1980, *T. Ahti & T. Goward*, H; Vancouver Island, Sproat Lake, c., 15 Km W of Port Alberni, 16-VI-1984, *T. Ahti*, H; Manitoba: Clearwater Lake Provincial Park (NE of The Pas), Sunset Beach Road, near SW corner of Day Lake, 25-V-2004, *T. Ahti, M. Piercey-Normore & T. Booth*, H; Northwest territories: District of Mackenzie, Nahanni National Park, Rabbitkttle Lake, 25-VII-1975, *G. W. Scotter, A. H. Marsh*, H; Mackenzie District, Eskimo lakes, 30-VII-1966, *G. W. Scotter*, H; Vicinity of Glacier Lake, Brintnell Lake, VII-1939, *L. Raup*, H; Saskatchewan: 11.9 miles southeast of Peerless on higway 26, westrn Saskatchewan, 25-VI-1971, *S. Tucker*, H; Yukon: Teslin Lake, 11-VI-1978, *P. Nimis*, H; Watson Lake town, by centenarial Avenue, 4-VII-1977, *T. Ahti*, H; 22-V-1957, *J. Comman*, L 794538; **Finland**: Central Finland: Saarijärvi, kk., poljetulla piennarniityllä, 23-IX-1959, *T. Ahti*, H; Eastern Uusimaa: N. Sibbo, Hangelby, Träskby, 31-V-1973, *R. Skytén*, H; North Karelia: Nurmeksen mlk., SE-osa, Mätäsvaarasta n. 2 Km W., 28-VII-1965, *J. Suominen*, H; Pielisjärvi, Kylänlahdesta n. 1 Km itään, 27-VII-1965, *J. Suominen*, H; Polvijärvi, Sola, Paljakka, adandoned mine, 27-IX-2003, *T. Ahti*, H; Northern Savonia: Jäppilä, Niinimäki, 16-VI-1990, *V. Haikonen*, H; Joroinen, Huutokosken asemasta n. 1.5 Km Joroisiin, 22-VII-1965, *J. Suominen*, H; Pieksämäen mlk., Haapakosken ratapihan S-osa, 1-VIII-1965, *J. Suominen*, H; Kiuruvesi, Niemisjärvi, prope viam ferr., 8-VIII-1960, *I. Huuskonen & P. Alanko*, H; Päijänne Tavastia: Hollola, Paimelanvuori, 10-IV-2008, *V. Haikonen*, H; Jyväskylän mlk., Kuohun, 13-VIII-1966, *J. Suominen*, H; Virrat, Virtain, 15-VIII-1964, *J. Suominen*, H; Kangasala, Pitkäjärvim Ilkko, 18-X-1986, *M. Kääntönen*, H; Pirkanmaa: Lempäälä, Aimala, 2-V-1986, *M. Kääntönen*, H; Lempäälä, Lastustenkulma, 2-V-1999, *M. Kääntönen*, H; Sr. Karkku, Kirkon vieressä, *J. Suominen*, H; South Karelia: Laatokan Karjala, Parikkala Joensuu, Taplavaara, adandoned mine, 13-IX-2003, *T. Ahti*, H; Virolahti, Ravijoki, peninsula Siikasaaari, 29-VI-1994, *T. Ahti & H. Bültann*, H; Southern Savonia: Imatra, Karhumäki, Karhumäenkadun, tasoristeyksestä, 23-IX-1994, *E. Thurén*, H; Kerimäki, Silvolan ratapihan W-pään N-reuna, 18-VII-1967, *J. Suominen*, H; Lappeentanta, Harapainen, Hietalankatu by railway crossing, 14-IX-1999, *T. Ahti*, H; Lappeentanta, Nieminen, NE of Ahola, 2-VI-1986, *O. Vitikainen*, H; Luumäki, Huopainen, 5 Km N of Taavetii, 29-VI-1994, *T. Ahti, F. J. Daniëls & H. Bültmann*, H; Valkeala, Selänpää, Honkala, 18-V-1986, *M. Kuusinen*, H; Tavastia Proper: Asikkala, Kalkkisen kanava, 2-IV-2008, *V. Haikkonen*, H; Asikkala, Kirkonkylä, Päijännetunnenlin läjitysalue, 10-X-2007, *V. Haikkonen*, H; Asikkala, Vääksy, Aurinkovuori, 4-V-2008, *V. Haikkonen*, H; Hollola, H; Kangasala, Ponsa, Pyyvuori, lähellä, *R. Mikkola*, H; Litti, Jokue, Ojala, 28-XI-2006, *V. Haikonen*, H; Nastola, Pyhäntaka, betoniliikken, 9-X-2007, *V. Haikonen*, H; Padasjoki, Kaurate, Mertsalmi, 17-VIII-1986, *T. Ahti*, H; Uusimaa: Artjärvi, Metsäkulma, Tähtikallio, 25-V-2008, *V. Haikonen*, H; Elimäki, Moisio, 1 Km SSW of Moisio manor, 27-IX-1991, *T. Ahti*, H; Helsinki, Malmi, Tattarinharju, 6-VI-1948, *L. Fagerström*, L 794539; Nurmijärvi, Kiljavan aseman, *M. Haapasaari*, H; Orimattila, Tekemäjärvi, 30-X-2003, *V. Haikonen*, H; Routsinpyhtää (Strömfors), Tesjoki, 23-V-1987, *T. Ahti*, H; **France**: Ródano-Alpes: Isère, Col d'Oinon, 11-X-1977, *Y. Rondon*, H; Alliola, Sudenpensänmäki, 9-VIII-2008, *V. Haikonen*, H; **Germany**: Hessen: Kreis Wetzlar, Dorf Niederkleen, kurzrasig-steinig-na ckterdige Sohle einer kl. Steingrube, 7-VIII-1976, *J. Futschig*, H;

Untermainebene Frankfurt, Eisenbahndamm, 1865, *S. A. Metzler*, FR 73890; Untermainebene Frankfurt, Eisenbahndamm, 1865, *S. A. Metzler*, FR 73889; Untermainebene Frankfurt, Eisenbahndamm, 1865, *S. A. Metzler*, FR 73888; West-Taiunus, Aubachtal Felsen an der Landstrasse östl. V. Niederlibbach Hunsrückschiefer, 1-VIII-1993, *H. Schöller*, FR 73887; Mecklenburg: Bei Schwerin, *Wüstnei*, H; Upper Bavaria: Auf bemoostem Boden der mit Gebüsche, Wolfratshausen, München, X-1893, *Arnold*, H; **Hungary**: Heves: Heves, 1913, *F. Fóriss*, L 794542; **Iran**: East Azerbaijan: Azebaijan-Tabriz-Jolfa toward to Khodaafrim-Missan Village, 15-VII-2001, *M. Sohrabi*, H; **Japan**: Hokkaido: Iburi, Lakeside of Utonaito, 29-X-1979, *H. Kashwadani*, H; Kamikawa, Kamikawa, Mr. Daisetsu Nature Park, Mt. Kurotake, 11-VIII-1970, *A. Kopone & T. Koponen*, H; Kam durch die Sammlung, *F.- Camus*, FR 55692; **Kazakhstan**: Vostochno-Kazakhstanskaja: 5 Km SSE of the ferry berth, 11-VI-1993, *R. Moberg & A. Nordin*, H; **Korea**: Ryanggang: 30 Km NE of Mt. Chang Bai at the connexion of Korea border with the border of Chinese An-tu and Hu-liong counties, 23-IX-1981, *T. Koponen*, H; **Netherlands**: Eiesland, Boschplant, IX-2002, *H. V. Dollan*, H; Tescheching, X-2002, *T. Kelnen*, H; **Norway**: Nord-Trondelag: Lierne, SE end of lake Tunnsjoen, between the road and the lake, 11-VIII-2004, *T. Tonsberg*, BG L79658; **Portugal**: Trás-Os-Montes: Rebordaos, Sª da Nogueira, 6-IX-2006, *A. R. Burgaz*, MACB 93984; **Russia**: Archangelsk-Oblast: Verkn, Pesha, 7-VII-1891, *A. O. Kihlman*, H; Grub. Archengelsk, Verkhn, Pesha, 28-VI-1891, *A. O. Kihlman*, H; Irkutsk: Siberia, Bratsk, shore of Bratsk sea, 20-VIII-1987, *J. Derome*, H; Karelia Republic: Kol Place, Vepskaya volost, N part of Sheltozero village, 28-IX-2004, *M. A. OapeeBa*, H; Severo-Primorsky-Park, Kurortny District in vicinity of Lisij Nos and Olgino, the south-east corner of the protected territory, *L. Konoreva & I. Stepanchikova*, H; Krasnoyarsk: N of Central Siberia, Severnaya Zemlya Archipelago, NW extremy of Bol'shevik Is., Ostantsovaia River canyon at 2 Km above its mouth at Mikoyan Bay, 12-VII-1996, *M. Zhurbenko*, H; Leningrad: Lodeynoe Pole District, Lodeynoe Pole, 2 Km E of the Svir Bridge, 17-VI-1991, *T. Ahti*, H; Sakha Republic: Yakutia, Khangalasskiy Dist., 5 Km S of Tabaga, above steep riverbank Tabaginskiy utës on W bank of Lena River (opposide to Khaptagay), 30-VI-2002, *T. Ahti*, H; Yakutia, Khangalasskiy Dist., Elanka (Yelanskoye), ca. 5 Km WNW of village centre, 19-VIII-2005, *T. Ahti & P. A. Timofeev*, H; Tuva Republic: South Siberia Mountains, Todginskaya Valley, central part of Azas Lake, 15-VIII-1997, *T. N. Otnyukova*, H; South Siberia Mountains, Todginskaya Valley, 14-VIII-1999, *T. N. Otnyukova*, H; **Spain**: Asturias: Pendones, 22-VIII-2005, *R. Pino-Bodas*, MACB 102908; Ávila: Ojos-Albos, 5-XI-2005, *A. R. Burgaz*, MACB 92821, MACB 93018; Barcelona: Montseny, Parc natural del Montseny, Turó de l'Home, 16-VIII-2006, *A. R. Burgaz*, MACB 94207; Cantabria: FuenteDé, 11-IV-1991, *A. R. Burgaz*, MACB 45288; Segovia: Cedillo de la Torre, 29-VI-1984, *A. R. Burgaz & M. Ventureira*, MACB 14182; Soria: Vozmediano, 4-IX-1984, *A. R. Burgaz*, MACB 45289; Teruel: Monterde, 13-VI-1991, *A. R. Burgaz*, MACB 45287; Valdelinares, estación de esquí, Sª de Gudar, 13-VI-1991, *A. R. Burgaz*, MACB 45292; Gerona: Puigcerdá, coll de Tosses, 18-VIII-2006, *A. R. Burgaz*, MACB 94210; Queralbs, Vall de Núria, 18-VIII-2006, *A. R. Burgaz*, MACB 94025; San Martín de Ogassa, mirador coll de la Torre, 17-VIII-2006, *A. R. Burgaz*, MACB 94206; Granada: Canilles, Sª de Baza, P. Nat. Sª de Baza, 12-V-2004, *A. R. Burgaz*, MACB 92995, MACB 102101, MACB 102102, MACB 102103; Lugros, Sª Nevada, Parq. Nac. de Sª Nevada, cabecera del río Alhama, 21-IV-2006, *A. R. Burgaz*, MACB 92996; Lugros, Sª Nevada, parq. Nac. de Sª Nevada, cabecera del río Alhama, 21-IV-2006, *A. R. Burgaz*, MACB 102889; Guadalajara: Peñalen, Alto Tajo, 16-V-2008, *A. R. Burgaz*, MACB 102890, MACB 102891; Lérida: Pas de la Casa, coll de la Bonaigua, 20-VIII-2006, *A. R. Burgaz*, MACB 102904, MACB 94208; Navarra: Balneario de Panticosa, Panticosa Ibón de Baños, 5-IX-1991, *T. Ahti & A. R. Burgaz*, MACB 45265; Elzaburu, 9-IX-1991, *T. Ahti & A. R. Burgaz*, MACB 45264; Palencia: Areños, Puerto de Piedras Luengas, 12-IV-1991, *A. R. Burgaz*, MACB 45291; **Sweden**: Gästrikland: Hille sn, Iggön, 11-V-2003, *G. Odelvik*, S L50027; Lappland: Åsele Lappmark, Vilhelmia, 30-I-1918, *C. Stenholm*, L 794541; Sverige: Pite lappmark, Arjeplog sn, 4100 m SSV om Bergviken. 3200 m. NV om Luttun. SO om Gárgok, toppen, S-sluttning, strax O om bäck, 24-VIII-2006, *G. Odelvik 0605*, S F59954; Uppland: Älvkarñeby par., Hyttön, 27-IV-1986, *A. Nordin*, UPS L-133603; Västerbotten: Bygdeå, Prästskär, S side, 28-VI-2004, *E. Eriksson*, UPS L-162988; **United Kingdom**: England: West Norfolk, Wendling, 21-XII-1958, *E. A. Ellis*, L 794540; **United States**: Michigan: Gogebic County, Ottowa National Forest, S of Yondota Falls along USFS 523, 9-VII-2004, *Clifford M. Wetmore No 90811*, S F53033.

***Cladonia cenotea* (Ach.) Schaer.**

Denmark: North Zealand: Hillerod, Tisvildeleje, Woodland area Tisvilde SW of town, 14-III-2009, *J. Vondrák* 6965.

***Cladonia cervicornis* (Ach.) Flot. *in* Jahresber**

Portugal: Alto Alentejo: Evoramonte, 10-I-2004, *A. R. Burgaz*, MACB 90695; Montinho, P. Nat. Sao Mamede, 11-I-2004, *A. R. Burgaz*, MACB 90696; Pégoes, Monte das Piçarras, 09-I-2004, *A. R. Burgaz*, MACB 91633; Beira Alta: Viseu, 26-I-1996, *A. R. Burgaz & I. Martínez*, MACB 66838; Beira Litoral: Sª do Açor, Fraga de Pena, Barroco de Degrainhos, 1.5 km de Benfeite, 29-III-1998, *T. Ahti & A. R. Burgaz*, MACB 68515; Viseu, San Miguel, 26-I-1996, *A. R. Burgaz & I. Martínez*, MACB 66838; Estremadura: Fernao Ferra, Reserva da Mata Nacional dos Medos, 07-VIII-1999, *A. R. Burgaz*, MACB 91628; Marinha Grande, Pinhal de Leiria, 17-VIII-1994, *A. R. Burgaz*, MACB 66839; Sª de Sintra, Monasterío de Peninha, 10-I-2004, *A. R. Burgaz*, MACB 90693; Sintra, Serra de Sintra, subiendo a la alta cruz en el Castelo dos Mouros, 16-III-1997, *A. R. Burgaz*, MACB 66956; Marinha Grande, Pinhal de Leiria, 17-VIII-1994, *Burgaz*, MACB 66839; Minho: Entre Ambos os Rios, 16-VI-1995, *Burgaz et al.*, MACB 66842; Sª de Peneda, Branda da Bouca dos Homens, 17-VI-1995, *Burgaz et al*., MACB 66841; Sapiaos, 12-VIII-1994, *Burgaz*, MACB 66840, MACB 66841; Serra do Gerês, Campo do Gerês, ribeiro do Sarilhaó, 18-VI-1995, *A. R. Burgaz, I. Martínez & P. Navarro*, MACB 90981; Soajo, proximidades del río Lima, 17-VI-1995, *A. R. Burgaz, I. Martínez & P. Navarro*, MACB 66842; Trás-os-Montes: Rebordelo, 12-VIII-1994, *Burgaz*, H, MACB 66840; **Spain:** Ávila: Ojos-Albos, 5-XI-2005, *A. R. Burgaz*, MACB 91692; Casavieja, Fuentelecha, 09-XI-2003, *A. R. Burgaz*, MACB 90701; Navamojada, 10-VI-1992, *A. R. Burgaz*, MACB 91630; Piedrahita, 21-XI-2004, *A. R. Burgaz*, MACB 90956; Ramacastañas, 14-XI-2004, *A. R. Burgaz*, MACB 90953; Barcelona: El Bages, Castellfollit del Boix, Sª de Rubio, 20-V-1998, *A. R. Burgaz*, MACB 91621; Burgos: Covarrubias, Sª de Covarrubias, *A. R. Burgaz*, MACB 91620; Pesquera de Ebro, 24-VII-1999, *A. R. Burgaz*, MACB 90855; Cáceres: Casas de Miravete, Puerto de Miravete, 09-I-2004, *A. R. Burgaz*, MACB 90732; Robledollano, Sª del Cabazal, 3-I-1995, *G. Aragón & I. Martínez*, MACB 90983; Villar del Pedroso, Sª del Hospital del Obispo, 04-I-1995, *G. Aragón & I. Martínez*, MACB 61565; Cádiz: Alcalá de los Gazules, El Picacho, P. Nat. de los Alcornocales, 21-IX-2004, *A. R. Burgaz*, MACB 91631; Chiclana, 29SQB6030, *A. R. Burgaz*, MACB 91625; Sª del Niño, 01-III-1997, *A. R. Burgaz*, MACB 90961; Cantabria: Puerto de S. Glorio, 07-VI-1994, *A. R. Burgaz*, MACB 90851; Vega de Liébana, Porcieda, 30TUN6876, *A. R. Burgaz*, MACB 91632; Castellón: Artana, Sª de Espadán, 11-IV-2003, *A. R. Burgaz*, MACB 90964; Chóvar, Sª de Espadán, 11-IV-2003, *A. R. Burgaz*, MACB 90966; Ciudad Real: Albaladejo, Sª del Relumbrar, 05-II-2003, *A. R. Burgaz*, MACB 90853; Alcolea de Calatrava, 04-VIII-1999, *R. I. Fernández & F. J. Sarrión*, MACB 86864; Almodóvar del Campo, Sª Umbría de Alcudia, puerto de San Juan, 03-II-1997, *A. R. Burgaz*, MACB 90975, MACB 80265; Fuencaliente, 04-I-1990, *F. Sarrión*, MACB37205; Fuencaliente, cuenca alta del río Cereceda, 29-I-1990, *A. R. Burgaz, E. Fuertes & F. Sarrión*, MACB 37204; Fuencaliente, Sª Madrona, río

Cereceda, 04-I-1990, *A. R. Burgaz*, MACB 90977; Fuencaliente, Aº Robledillo de las Hoyas, 05-II-1997, *A. R. Burgaz, I. Martínez & F. J. Sarrión*, MACB 90977; Puebla de D. Rodrigo, 16-VI-1993, *A. R. Burgaz*, MACB 90972; Puebla de Rodrigo, carreterín del repetidor de la Celadilla, 23-II-1997, *R. I. Fernandez & F. J. Sarrión*, MACB 86865; Saceruela, Sª de Canalizos, 24-IX-2004, *A. R. Burgaz*, MACB 90973; Solana del Pino, río Robledillo, 26-X-1996, *R. I. Fernández & F. J. Sarrión*, MACB 86863; Villarrubia de los Ojos, Sª de la Cueva, Pto. de los Santos, 05-XI-2004, *A. R. Burgaz*, MACB 90974; Córdoba: Fuente Obejuna, Valdeinfierno, 23-IV-2006, *A. R. Burgaz*, MACB 92691; Villaharta, fuente de la Lastrilla, 24-IX-2004, *A. R. Burgaz*, MACB 90716; Cuenca: San Clemente, 11-V-2004, *A. R. Burgaz*, MACB 90718; Guadalajara: Bustares. Sª de Alto Rey, 23-VIII-2003, *A. R. Burgaz*, MACB 90725; Cantalojas, barranco río Lillas, P. Nat. Tejera Negra, 23-VIII-2003, *A. R. Burgaz*, MACB 90720; Cardoso, *A. R. Burgaz*, MACB 90730; Gascueña de Bornova, Sª de Alto Rey, 23-VII-2003, *A. R. Burgaz*, MACB 90719; Monte Alcarria, 27-X-1970, SANT 1631; Huelva: Aracena, camino a Castanedo, 18-VII-1995, *A. R. Burgaz*, MACB 90728; Castaño del Robledo, Sª Aracena, 27-IV-1993, *I. Martínez*, MACB 91593; Matalascañas, 11-VIII-1996, *A. R. Burgaz*, MACB 91610; Huesca: Formigal, Barranco de Broncuso, 06-IX-1991, *T. Ahti & A. R. Burgaz*, MACB 46346; Panticosa, Ibón de Baños, 05-IX-1991, *T. Ahti & A. R. Burgaz*, MACB 46347; Jaén: Barranco de Valdeazores, P. Nat. de Despeñaperros, 9-V-2005, *A. R. Burgaz*, MACB 92686; Montizón del río Dañador, 06-II-2003, *A. R. Burgaz*, MACB 90832; Valdeazores, Despeñaperros, 15-XI-1993, *A. R. Burgaz*, MACB 90854; La Coruña: A Capelada, Cedeira, playa próxima a Obico, 26-VII-1993, *M. J. Sanchez-Biezma, G. Paz & M. E. López de Salines*, SANT 9040; Cabo de San Adrian, Ermita S. Adrián, 10-IX-1997, *G. Paz*, SANT 10297; La Rioja: Anguiano-Mansilla de la Sª, valle del río Najerilla, 08-IX-2004, *A. R. Burgaz*, MACB 89516; Lumbreras, 21-X-1983, *A. R. Burgaz & Mendiola*, MACB 14661; Mansilla, Barranco de Mansilla, 19-IX-1990, *A. R. Burgaz*, MACB 45681; Posadas, Sª de San Lorenzo, valle del río Oja, 07-IX-2004, *A. R. Burgaz*, MACB 89734; León: Colinas del Campo de Martín Moro, 22-X-1994, *A. R. Burgaz*, MACB 91623; Lugo: Fonsegrada, Vilarin de Abaixo, 10-II-1987, *C. P. Valcárcel*, SANT 17294; Gundriz, Minicipio Samos, valle de Louzara, 24-III-2005, *A. Noguerol Seoane*, MACB 92687; Madrid: Arganda, 31-I-1982, *A. R. Burgaz*, MACB 37203; Cervera de Buitrago, 26-VI-1991, *A. R. Burgaz*, MACB 45680; Ctra. Rascafría al Puerto de Navacerrada, 13-XI-1994, *G. Aragón, J. Castillo, A. Herrero, I. Martínez*, MACB 90839; El Cuadrón, 21-VI-1997, *A. R. Burgaz & S. Casas*, MACB 75257; Embalse de Ríosequillo, 21-III-1997, *A. R. Burgaz & S. Casas*, MACB 75228; Hoyo de Manzanares, 01-XI-1989, *A. R. Burgaz*, MACB 37210; Monte de El Pardo, 09-IV-2004, *A. R. Burgaz*, MACB 90840; Torrelaguna, 21-III-1997, *A. R. Burgaz & S. Casas*, 75238; Navarra: Esteribar, valle de Baztán, Barranco Olazar, 08-IX-1991, *A. R. Burgaz & T. Ahti*, MACB 90841; Eugui, 08-IX-1991, *T. Ahti & A. R. Burgaz*, MACB 46348; Orense: Barranco do Banho, XI-97, MACB 70267; Montes do Invernadeiro, Villarino de Conso, 19-VI-1995, *A. R. Burgaz*, MACB 90979; Pontevedra: Islas Cies, 27-V-1996, *G. Paz*, SANT 10296; Moaña, Mirador de Faro Domaio, 01-IX-2002, *A. R. Burgaz*, MACB 91622; Salamanca: Beleña, 5-XI-2005, *A. R. Burgaz*, MACB 91657; El Cabaco. Sª de la Peña de Francia, 6-XI-2005, *A. R. Burgaz*, MACB 91691; Frades de la Sª, Sª de Frades, 5-XI-2005, *A. R. Burgaz*, MACB 91661; Horcajo de Montemayor, 26-IX-1991, *A. R. Burgaz*, MACB 45682; La Alberca, Sª de las Mestas, Pº del Portillo, 6-XI-2005, *A. R. Burgaz*, MACB 91671; La Alberca, Sª de las Mestas, valle del río Batuecas, 6-XI-2005, *A. R. Burgaz*, MACB 91672; Las Mestas, Sª de las Mestas, valle del río Batuecas, 6-XI-2005, *A. R. Burgaz*, MACB 91673; Monsagro, Sª de la Peña de Francia, Paso de los Lobos, 6-XI-2005, *A. R. Burgaz*, MACB 91666; Segovia: Aguilafuente, 14-XI-1993, *A. R. Burgaz*, MACB 91627; Sevilla: Alanis, P. Nat. de Sª Norte, Mirador Loma del Aire, 23-IV-2006, *A. R. Burgaz*, MACB 92692; Cazalla de la Sª, Parq. Nat. de la Sª Norte, finca UPA, 23-IV-2006, *A. R. Burgaz*, MACB 92693; Cazalla de la Sª, Sª de Grana, Parq. Nat. de la Sª Norte, 24-IV-2006, *A. R. Burgaz*, MACB 92697; Soria: Ágreda, 04-IX-1984, *A. R. Burgaz*, MACB 45675; Alto de Villaciervos, 03-V-1999, *A. R. Burgaz, M. A. Carrasco & E. Fuertes*, MACB 91606; Lubia, Altos de Lubia, 07-IX-2003, *A. R. Burgaz*, MACB 90843; Matalebreras, 10-IX-1991, *T. Ahti & A. R. Burgaz*, MACB 45673; Ólvega, 1070 m, *A. R. Burgaz*, MACB 45275; Vozmediano, 04-IX-1984, *A. R. Burgaz*, MACB 45676; Teruel: Albarracín, 12-VI-1991, *A. R. Burgaz*, MACB 45674; Toledo: Belvís de la Jara, 30-IX-2005, *R. Pino-Bodas*, MACB 91595; Robledo del Mazo, 15-II-2006, *R. Pino-Bodas*. Zamora: La Tabla, 06-X-1997, *G. Aragón, A. R. Burgaz & A. Terrón*, MACB 70243; Pereruela, arroyo del Zape, 07-IX-1998, *A. R. Burgaz, S. Casas & I. Rodríguez de Lope,* MACB 70244; Zaragoza: Sestrica, 20-IX-2003, *A. R. Burgaz*, MACB 90847; Sestrica, Sª de la Virgen, 29VI-1988, *A. R. Burgaz*, MACB 45679; Vera de Moncayo, Sª de Moncayo, 13-IX-2003, *A. R. Burgaz*, MACB 90848.

***Cladonia coniocraea* (Flörke) Spreng.**

Andorra: Les Escaldes-Engordany, hacia collado de Jovel, 6-XII-1994, *A. Herrero & I. Álvarez*, MACB 94412; Les Escaldes, Engordany, 6-XII-1994, *A. Herrero & I. Álvarez*, MACB 94615; Ordino, coll de Ordino, 19-VIII-2006, *A. R. Burgaz*, MACB 94363; Vall d'Incles, hacia el refugio de Sisqueró, 7-XII-1994, *A. Herrero & I. Álvarez*, MACB 94434; **Australia**: New South Wales: South Coast, west-facing road cut, Merimbula Tathra road, 5 Km N of Merimbula, V-1998, *S. Hammer*, CANB 619971; New South Wales: South Coast, west-facing road cut, Merimbula Tathra road, 5 Km N of Merimbula, V-1998, *S. Hammer*, CANB 619986; Southerm Tablelands, Budawang Montain, Morton National Park, Braidwood-Nowra Road, 42 Km NE of Ulladeulla, I-1998, *S. Hammer*, CANB 619997; Southerm Tablelands, Morton National Park, Garbage dump between Sassafras and Tianjarra Falls, 31 Km NE of Ulladulla, V-1998, *S. Hammer*, CANB 619996; Victoria: Gippsland, Errindura National Park, Errindura Saddle, 20 Km SSE of Bonang, V-1998, *S. Hammer*, CANB 619970; Western Australia: Darling, Ridge wirh bare-littered, 2 Km S of Quinninup brown sand clay loam, 3-VII-1999, *R. J. Cranfield*, CANB 658367; **Austria**: Estiria (Steiermark): Alpes orientales, Nördliche Kalkalpen, Sª de Hochschwab, valle de Siebenseebach sur del pueblo Wildalpen, 20-IX-1996, *A. R. Burgaz, J. Hafellner & I. Martínez*, MACB 65994; Steirisches Randgebirge, koralpe, valle del río Laβnitz (Klause), W de la ciudad Deutschlandsberg, 15-IX-1996, *A. R. Burgaz, J. Hafellner & I. Martínez*, MACB 65993; **Belgium**: Walloon: Namur Viroinval, Treignes, 2-V-1999, *L. Spier*, L 753073; **Bulgaria**: Sofia province: Borovetz, 21-VII-1993, *A. R. Burgaz*, MACB 94424; **Canada**: Manitoba: SE of Flin Flon, Thompson Mine, Little Spruce Lake Road, 24-V-2004, *T. Ahti & M. Piercey-Normone & T. Booth*, H; Nova Scotia: Colchester Co., Economy River Wilderness area, vicinity of Economy Falls, at N end River Phillip Road, ca. 8 Km N of Hwy 2, 14-V-2004, *T. Ahti*, H; Cumberland Co., Cape Chignecto Provincial Park, 14-V-2004, *T. Ahti*, H; **Chile**: Región XII, Magallanes y Antártica Chilena: Isla de Navarino, Lago Róbalo, 18-I-2005, *R. Vilches*, MACB 91997; Isla de Navarino, subida al cerro de la Bandera, mirador, 15-I-2005, *A. R. Burgaz*, MACB 91992; Isla de Navarino, caleta Wulaia, 23-I-2005, *R. Vilches*, MACB 91999; Isla de Navarino, Cerro Róbalo, 18-I-2005, *R. Vilches*, MACB 91996; Isla de Navarino, camino de Lum, 26-I-2005, *R. Vilches*, MACB 91998; Puerto Natales, río Serrano, 30-I-2005, *A. R. Burgaz*, MACB 92207; Isla de Navarino, Puerto William, valle del río Ukika, camino a Media Luna, 18-I-2005, *A. R. Burgaz*, MACB 91994; Isla de Navarino, Dientes de Navarino, 19-I-2005, *Popi*, MACB 91995; **China**: Xinjiang: Tianshan Mountains, Gongliu, 1999, *A. Abbas*, FH; **Cuba**: Santiago de Cuba: Sª de la Gran Piedra, pasado la Isabelica, camino arroyo, 31-VII-2007, *A. R. Burgaz & D. Rosabal*, MACB 1028520; Sª de la Gran Piedra, subida a la Gran Piedra, 30-VII-2007, *A. R. Burgaz & D. Rosabal*, MACB 102980; **Finland**: Uusima: Sibbo, Skogsby, Storstenkläven, 20-VI-1990, *T. Ahti, A. R. Burgaz & Fuertes*, MACB 50785; Central Finland: Keuruu, Haapamäki, Tiusala, E side of Tyrisevänjärvi, 17-V-1959, *T. Ahti*, H; Central Ostrobothnia: Kaarlelan alue, Saarukan SE-puol, 23-V-1967, *K. Takala*, H; Kälviä kk., aseman lähellä, 19-IV-1967, *K. Takala*,

H; Kälviä, 2 km kkästä Ridan Kylän, 14-IV-1967, *K. Takala*, H; Kälviä, Umpisuonmäen NE-puol, 5 Km Ridan Kylän, 27-XI-1966, *K. Takala*, H; Kälviä, Umpisuonmäen NE-puol, 5 Km Ridan Kylän, 27-XI-1966, *K. Takala*, H; Kannus, Jouhineva, 1-VIII-1979, *R. Skytén & K. Silfverberg*, H; Finland Proper: V. Nagu, island Själö, SE part, 24-IX-1983, *T. Ahti*, H; V. Nagu, island Själö, W of Turku University Research Station, 25-IX-1983, *T. Ahti*, H; Vihti, Ollila, Vaakkoi Recreation area, SE of Lake Vaakkoi, 19-VI-1995, *S. Hyvönen & T. Ahti*, H; Kymenlaakso: Hogland, Kotikallio, 1-VIII-1868, *M. Brenner*, H; Vahkalahti, Ulko-Tammio, 1930, *V. Krohn*, H; Virolahti, Säkjärvi, Onkamaanjoki, 12-IX-2008, *V. Haikonen*, H; Lapland: Sompion Lappi, Sodankylä, site of Lokka Reservoir, 18-VI-1959, *T. Ahti*, H; Tornio, Pensaskari, 4-X-1992, *U. Nummela-Salo & P. Salo*, H; Northern Ostrobothnia: Hailuoto, Pöllänojasta, 1 Km Sunikariin päin, 15-VII-1961, *A. Koponen*, H; Kuusamo, ad Kutsajoki, Nivajärven W-rantamutkan ja Hirveäkallion väl, 25-VII-1937, *M. Laurilla*, H; Kuusamo, Juuma, Jäkälävuoma, 6-VII-1981, *R. Skytén*, H; Kuusamo, Virkkula, Porontimajoki-Myllylampi, 7-VII-1981, *R. Skytén*, H; Pudasjärvi, Sotkjärven Kylän, 29-VIII-1965, *K. Takala*, H; Utajärvi, Kaihlasperä, Kaihlanen, 4-VIII-1974, *P. Uotila & H. Toivonen*, H; Päijänne Tavastia: Hämeenkoski, Ilolanharjut, 21-VIII-2008, *V. Haikonen*, H; Heinola, Mäyrämäki, 8-XI-2005, *V. Haikonen*, H; Hollola, Hankaa, Kotomäki, 9-IV-2005, *V. Haikonen*, H; Hollola, Kalliola, Sudenpänmki, 9-VIII-2008, *V. Haikonen*, H; Iitti, Haapataipale, Selkäapajanmäki, 16-VI-2008, *V. Haikonen*, H; Iitti, Jokue, Ojala, 28-XI-2006, *V. Haikonen*, H; Iitti, Vuolenkoski, Isomäki, 25-X-2008, *V. Haikonen*, H; Iitti, Vuolenkoski, Sahanniemi, 25-X-2008, *V. Haikonen*, H; Janakkala, Rehakka, Järvenpää, 2-VI-1969, *P. Uotila*, H; Längelmäki, Löytäne, Harjunsivu-talon, 12-VI-1987, *L. Miekk-oja*, H; Loppi, Puhälampi, Kerminotko, 3-V-2003, *V. Haikonen*, H; Padasjoki: kirkonkylä, Koiravuori, 11-III-2008, *V. Haikonen*, H; South Karelia: Imatra, Savikanta, ala-asteelta, 19-III-1995, *E. Thurén*, H; Juva, Kaihunmäki, Halla-aho, 2-XI-1987, *O. Vitikainen*, H; Juva, Kaihunmäki, W of Halla-aho, 22-X-1988, *O. Vitikainen*, H; Parikkala, Kinnarniemi, Koskutjoki Lövsly, 5-VIII-1979, *R. Skytén & K. Silfverberg*, H; Taipalsaari, Karhunpää, 5-X-1969, *O. Vitikainen*, H; Pirkanmaa: Kuru commune, The National Park of Seitseminen, Multiharju-Seitsemisjoki, 22-VI-1989, *S. Laaka*, H; Kuru commune, The National Park of Seitseminen, Multiharju-Seitsemisjoki, 22-VI-1989, *S. Stenroos*, H; Vesilahti, Kirveslammi, Vuolisjärvenvuori, 5-X-2002, *M. Kääntönen*, H; Satakunta: Satakunta, Kolemäki, Kynsikangas, 2 Km NW of Plättilänmaa road junction, 13-V-1959, *T. Ahti*, H; Tavastia Proper: Hämeenlinna, Vanaja, Katumajärvi, Mantereenvuori, 18-I-1975, *K. Oittinen*, H; Nokia, Siuro, Uusikulä, Ketaranvuori, 8-XI-1997, *M. Kääntönen*, H; South Häme, Kalvola, Kónnölä, Kaivolampi, 18-IV-2008, *H. Väre*, H; South Häme, Kuru commune, The National Park of Seitseminen, Multiharju, 22-VI-1989, *S. Laaka*, H; Tammela, ca 3.5 Km om Liesjärvi nationalparks, 13-VI-1982, *R. Skytén & K. Silfverberg*, H; Uusimaa: Artjärvi, Metsäkulma, Tähtikallio, 25-V-2008, *V. Haikonen*, H; Askola, Kirkonkylä, Vanhainkoti, 7-V-1988, *T. Ahti*, H; Esbo, Bolarskog, 17-VIII-1985, *R. Skytén*, H ; Finland: Uusimaa: Espoo, Mäkkylä, 10-X-1957, *B. Saltikoff*, H; Espoo, SW end of Kattilajärvi, 9-IX-1988, *T. Ahti*, H; Helringuav, 1858, *W. Nylander*, H; Helsinki, Kaisaniemi, University Botanical Garden, 21-V-1970, *T. Ahti*, H; Helsinki, Kaisaniemi, University Botanical Garden, 23-VIII-1970, *H. Vänskä*, H; Helsinki, Suomenlinna, 22-V-1983, *J. Issakainen*, H; Uusimaa: Kirkkonummi, Hirsala by Väransby road, ca. 0.5 Km from the Hirsala bridge, 3-X-1970, *T. Ahti*, H; Mäntsälä, NE-osa, Hunttijärven, 22-V-1966, *J. Suominen & T. Vuori*, H; N. Mäntsälä, Kirkonkylä, Järvimäki, 4-VIII-1949, *L. Korhonen*, H; Nurmijärvi, Klaukkala, Tornimäki, S slope, 14-I-1983, *T. Ahti & B. Tan*, H; Nurmijärvi, Perttula, Äijänkallio, 29-IX-1995, *T. Ahti, A. R. Burgaz, I. Martínez & O. Vitikainen*, MACB 96299; Orimattila, Kaitala, Kairesuonkangas, 20-XI-2005, *V. Haikonen*, H; Sibbo, Gumbostrand, Mosshälla, 16-V-1969, *A. Nordström*, H; Sibbo, Kollbäck, 1-V-1997, *T. Ahti*, H; Sibbo, Skogsby Storstenkläven, 20-VI-1990, *T. Ahti, A. R. Burgaz & E. Fuertes*, MACB 50797; Sibbo, Skogsby Storstenkläven, 20-VI-1990, *T. Ahti, A. R. Burgaz & E. Fuertes*, MA-Lichen 4581; Tuusula, Ruotsinkylän kokelaulue, 4-VI-1957, *T. Ahti*, H; Porvoo rural parish, island Emäsalo, Varlaxudden, 10-X-1995, *T. Ahti, A. R. Burgaz, I. Martínez & O. Vitikainen*, MACB 96311; South Karelia: Imatra, Lammassaari, SE-ranta pastotiestä, 16-IV-1993, *E. Thurén*, H; **France**: Champagne-Ardenne: Ardennes Chooz, 30-IV-1999, *L. Spier*, L 753074; **Germany**: Bavaria: Angsburg, Haspehmarar, *M. Britzelmayr*, H; Oberbayern, Lkr. München, Forstenrieder Park, Ludwig-Geräumt, 5-II-1984, *T. Ahti & H. Hertel*, H; Baden-Württemberg: Karlsruhe: auf sonnigem, sandigen Boden in einem Kiefernwald zwischen Blankenloch und Leopolshafen, 16-IV-1967, *H. Schindler*, H; Lower Saxony: iedersavhsen, Olderburg, Olderburg Sand, 1918, *Sandstede*, H; Tongrube a/d B 51, Ostercappeln, 7-V-1995, *K. van Dort*, L 753002; Oldenburg, Am Waldrande unter Buchen und Tannen bei Gristede, X-1918, *Sanstede*, H; Oldenburg, An Föheren im Barneführer Holz, II-1920, *Sanstede*, H; Oldenburg, Auf faulenden Föhrenstrünken in Willbrook bei Zwischenahn, VII-1887, *H. Sandstede*, B 60 0058135; Oldenburg, Herrenneuen b. Varel, 17-IV-1936, *Langerfeldt*, B 60 0134299; Oldenburg, Rihtmor, 1889, *H. Sandstede*, B 60 0058154; Oldenburg., BegleitpflanzenMit Nr. 385 am Waldrande bei Gristede, X-1918, *H. Sandstede*, B 60 0058160; Oldenburg: Ad Willbrook prope Zwischenahn, *H. Sandstede*, B 60 0058207; Mecklenburg: Am Grunde von Föhren zusammen, 23-VI-1918, *Sandstede*, H; Jüngere Pfanze, 23-VI-1918, *Sanstede*, H; Rhineland-Palatinate: Willbrook, 1887, *H. Sandstede*, B 60 0058155; Thüringen: Am Rande lichten Föhrenfchonung bei Reinhardsrod, 1918, *Th. Reinstein-Schmalkelden*, H; Zollverein, 27-V-2003, *L. Spier*, L 753072; **India**: North India: Himachal Pradesh, Sirmaur District, Rajgarch, Habban Reserve, 30-V-2000, *S. Chatterjee, A. N. Dubey & S. Nayaka*, H; **Iran**: Golestan: Gorgan, Gorgan toward Zyiarat village aroung village, 14-V-2001, *M. Sohrabi*, H; Mazandaram: Nour, Kojdur and Kodir villagies, 3-IV-2002, *M. Sohrabi & M. Mofid*, H; Nour, Kojdur and Kodir villagies, 3-IV-2002, *M. Sohrabi & M. Mofid*, H; Nour, the road of Nour toward Arnol, Lavij village around the road, 2-IV-2002, *M. Sohrabi & M. Mofid*, H; **Italy**: Toscana: Frassigoni, IX-2000, *R. Benesperi*, H; **Luxenburgo**: Esch. S. Sûre, 3-IX-1955, *R. W. Becking*, L 794579; **Mongolia**: Buteliyn-Nuru Range, valley of river Barun-Atsyn-Gol, 27-VIII-1974, *N. S. Golubkova & U. Tsogt*, H; **New Zealand**: South Island: Highway 6 southbound, Westland vicinity of Nelson, XII-1998, *S. Hammer*, CANB 566024; Old Whangamon Hill Road, vicinity of Nelson, XII-1998, *S. Hammer*, CANB 566029; **Pakistan**: Azad Jammu and Kashmir: Loon Bagla, Muzaffarabad, 22-VII-1963, *Almad*, L 794576; **Portugal**: Beira Alta: Manteigas, Sª. da Estrela, P. Nat. de Sª da Estrela, cabecera del río Zêzere, 2-V-2007, *A. R. Burgaz*, MACB 94635; Bussaco, Cruz Alta, 3-IV-1998, *T. Ahti & A. R. Burgaz*, MACB 66932; Beira Litoral: Mealhada, Luso, Buçaco Cruz alta, 28-I-1996, *A. R. Burgaz & I. Martínez*, MACB 66922; Sª do Açor, Mata de Margaraca 2.5 Km de Benfeite,taludes, 29-III-1998, *T. Ahti & A. R. Burgaz*, MACB 66934; Sª do Açor, Mata de Margaraça, a 2.5 Km de Benfeite, 29-III-1998, *T. Ahti & A. R. Burgaz*, MACB 66921; Sª do Açor, Fraga de Pena, Barroco de Degrainhos, 1.5 Km SSE de Benfeite, 29-III-1998, *T. Ahti & A. R. Burgaz*, MACB 66933; Minho: Adrao, Gavieira, Sª de Soajo, 17-VI-1995, *A. R. Burgaz, I. Martínez & P. Navarro*, MACB 93811; Entre Ambos os Rios, 17-VI-1995, *A. R. Burgaz, I. Martínez & P. Navarro*, MACB 94433; Soajo-Adrao, Sª do Soajo, 17-VI-1995, *A. R. Burgaz, I. Martínez & P. Navarro*, MACB 94432; Trás-Os-Montes: Alrrededores de Sª da Serra, Sª de Nogueira, 20-II-2005, *A. R. Burgaz*, MACB 94343; Rebordainhos, Sª de Nogueira, 19-II-2005, *A. R. Burgaz & J. Marques*, MACB 94703; Rebordainhos, Sª de Nogueira, 19-II-2005, *A. R. Burgaz*, MACB 94342; Azores Islands: Flores, Strassenzufahrt Faja Grande, 7-VIII-2003, *F. Bergen*, H; Pico, 2002, *C. Freitas*, H; Pico, Sao Joao, 2003, *A. F. Rodrigues*, H; Sao Jorge, 2002, *C. Freitas*, H; Sao Jorge, Topo, 2003, *A. F. Rodrigues*, H; Sao Miguel, 2002, *C. Freitas*, H; Sao Miguel, Ponta Delgada, 2003, *A. F. Rodrigues*, H; Terceira, Canada Francesa, 2004, *A. F. Rodrigues*, H; Terceira, Furnas do Enxofre, 2003, *A. F. Rodrigues*, H; Terceira, Furnas do Enxofre, 2003, *A. F. Rodrigues*, H; **Russia**: Karelia Republic: East Pomoria, Belomorskii District, 19-VIII-2002, *P. Uotila*, H; East Pomoria, Belomorskii district, Onezhskaya Guba, Kothano Island, 19-VIII-2002, *P. Uotila*, H; East Pomoria, Belomorskii district, Onezhskaya Guba, 19-VIII-2002, *P. Uotila*, H; Karelia (olonetsensis), Prionegra district,

Polovina (Posta, c. 20 Km NE of Pryazha), 24-VI-1991, *T. Ahti*, H; Karelia Pomorica occidentalis, Muyzero District, Androvaya gora, 16-VII-1996, *T. Ahti*, H; Transonega Karelia, Medvezh'egorsk district, Lake Onega, NE of Tolvuja, 3-VII-2004, *P. Uotila*, H; Leningrad Oblast: Hogland, Kellerkallio, 29-VIII-1868, *M. Brenner*, H; Murmansk: Kirovsk District, Khibiny Mts., in valley of river Kunijok, 3 Km NE of geological station, 11-VII-1998, *K. Kärkkäinen*, H; Lapponia Imandrae, Kirovsk District, Khibiny Mts., 11-VIII-1998, *K. Kärkkäinen*, H; Lapponia Imandrae, Kirovsk District, Khibiny Mts. In valley of river Kunijok, 2 Km NE of geological station, 10-VII-1998, *K. Kärkkäinen*, H; Lapponia Imandrae, Krovsk District, Khibiny Mts., in valley of river Kunijok, 2 km of geological station, 10-VII-1998, *K. Kärkkäinen*, H; Sakha Republic: Khangalasski District, Elanka, ca. 5 Km WNW of village centre, 19-VIII-2005, *T. Ahti & P. A. Timofeev*, H; Khangalasskiy District, Elanka, ca. 5 Km WNW of village centre, 19-VIII-2005, *T. Ahti & P. A. Timofeev*, H; Yakutsk, Municipality,ca. 40 Km NW of Yakutsk, 2 Km W of Spasskoe, N of Forestry Station, 7-VII-2002, *T. Ahti*, H; Sakhalin Oblast: Far east, Kurile Island, Kunashir Island, Andrevejska river, 28-VIII-2003, *S. Abrahamczyk*, H; Tuva Republic: South Siberia Mountains, Todginskaya Valley, State Reserve "Azas" east end of the Azas Lake, 18-VIII-998, *T. N. Otnyukova*, H; South Siberia Mountains, Todginskaya Valley, Tooro-Khem Village, Tooro Khem River in its low stream, 25-VII-1999, *T. N. Otnyukova*, H; **Slovakia**: Banská Bystrica: Montes Štiavnica vrchy, montis Kamenná supra pag. Vyhne, 7-VIII-1987, *I. Pisút et Svetlíkova*, BRA-CR 10016; Žilina: Sihelné, valley Dlhej vody near cottage to green tourist sign to mt. Pilsko, 19-VIII-2000, *V. Orthová*, BRA-CR 6250; **South Africa**: Cape Province: George-Wilderness, 1-XII-1949, *R. A. Maas Geesteranus*, L 794575; **Spain**: Albacete: Bogarra, Sª de Alcazar, cerca de Las Espineras, 6-X-1996, *G. Aragón, A. Herrero & A. Pujol*, MA-Lichen 9737; Riópar, Sª del Calar del Mundo, subida a la Fuente de la Pedorrilla, 5-I-1996, *G. Aragón & I. Martínez*, MA-Lichen 7288; Asturias: Cerredo, 21-X-1994, *A. R. Burgaz*, MACB 93617; Cerredo, Sª de Degaña, 24/10/1994, *A. R. Burgaz & I. Martínez*, MACB 94425; Cerredo, Sª de Degaña, 24-X-1994, *A. R. Burgaz & I. Martínez*, MACB 94413; Felechosa, Foces del Pino, 5-VIII-2003, *A. R. Burgaz*, MACB 93729; Felechosa, Foces del Pino, 29-I-1990, *F. J. Sarrión*, MACB 43863; La Raya, Reserva Nac. de Mampodre, Pto. de San Isidro, 22-VII-2006, *A. R. Burgaz*, MACB 102845; Páramo, Pto. de Ventana, 25-X-1994, *A. R. Burgaz*, MACB 94428; Salgueiras, 21-VIII-2006, *A. R. Burgaz*, MACB 102895; Salgueiras, robledal de Salgueiras, Villanueva de Oscos, 21-VII-2006, *A. R. Burgaz*, MACB 95397; Somiedo, Villar de Vildas, río Pigüena, 1-XI-1993, *G. Aragón, A. Herrero & I. Martínez*, MACB 94426; Tineo, 6-VIII-1995, *P. Navarro*, MACB 94427; Ávila: Candelario, P. Nat. Sª. de Candelario, subida a El Travieso, 1-VII-2007, *A. R. Burgaz*, MACB 95320; Casavieja, Fuentelecha, 9-XI-2003, *A. R. Burgaz*, MACB 93806; La Hoya, Alto de la Covatilla, 1-VII-2007, *A. R. Burgaz*, MACB 95319; Piedrahita, 21-XI-2004, *A. R. Burgaz*, MACB 102873; Piedrahita, 21-XI-2004, *A. R. Burgaz*, MACB 102874; San Bartolomé de Bejar, 1-VII-2007, *A. R. Burgaz*, MACB 95318; Barcelona: El Brull, Parc Natural del Montseny, coll de Formic, 16-VIII-2006, *A. R. Burgaz*, MACB 102850; Montseny, Parc Natural del Monseny, 16-VIII-2006, *A. R. Burgaz*, MACB 102847; Montseny, Parc Natural del Monseny, Turó de l'Home, 16-VIII-2006, *A. R. Burgaz*, MACB 102869; Burgos: Herramel, 2-V-1991, *A. R. Burgaz*, MACB 45968; Neila, Sª de Neila, 6-IX-2003, *A. R. Burgaz*, MACB 93809; Neila, Sª de Neila, 9-IX-2003, *A. R. Burgaz*, MACB 93618; Pesquera del Ebro, 8-VI-1988, *A. R. Burgaz*, MACB 45969; Quintanar de la Sª, 6-IX-2003, *A. R. Burgaz*, MACB 93800, MACB 93812; Santa Cruz del Valle Urbión, 2-IX-1991, *I. Martínez*, MACB 45963; Cáceres: Villar del Pedroso, garganta del Mesta, 4-I-1995, *G. Aragón & I. Martínez*, MACB 64896; Villarreal de San Carlos, P. Nat. de la Sª de Monfragüe, 4-V-2007, *A. R. Burgaz*, MACB 95089; Cantabria: Camaleño, P. N. P. E., FuenteDé, 15-XI-2003, *G. Amo, A. R. Burgaz, I. Martínez & M. Otalora*, MACB 89752, MACB 89753; FuenteDé, 11-IV-1991, *A. R. Burgaz*, MACB 45956; Camaleño, Invernales de Mato, 8-VI-1994, *A. R. Burgaz*, MACB 94421, MACB 94431; Camaleño, P. N. P. E., Cosgaya, 14-XI-2003, *G. Amo, A. R. Burgaz, I. Martínez & M. Otalora*, MACB 89744; Camaleño, P. N. P. E., Cosgaya, 15-XI-2003, *G. Amo, A. R. Burgaz, I. Martínez & M. Otalora*, MACB 89745; Campoo de Cabuerniga, río Saja, 1-IV-1994, *G. Aragón & I. Martínez*, MACB 93814; Campoo de Cabuerniga, río Saja, 31-III-1994, *G. Aragón & I. Martínez*, MACB 94420; Fuentedé, 6-VIII-1979, *E. Ron*, MACB 84681; Saja, 15-VIII-1991, *I. Martínez*, MACB 45972;Ciudad Real: Fuencaliente, arroyo del Robledo de las Hoyas, 5-II-1997, *A. R. Burgaz, I. Martínez & F. J. Sarrión*, MACB 80300; Fuencaliente, curso alto del río Cereceda, 29-I-1990, *F. Sarrión*, MACB 43871; Fuencaliente, curso medio del río Cereceda, 27-VI-1993, *F. J. Sarrión*, MACB 80299; Fuencaliente, pista Cereceda-Valmayor, 2-V-1997, *F. J. Sarrión*, MACB 80302; Fuencaliente, río Cereceda medio-Dornilleros, 27-VI-1993, *F. J. Sarrión*, MACB 80298; Fuencaliente, río Cereceda, 29-VI-1992, *F. J. Sarrión*, MACB 64875; Fuencaliente, Sª de Navalmanzano, 30-V-1998, *F. J. Sarrión*, MACB 80304; Fuencaliente, umbria de la Sª de Puerto Viejo, 16-V-1998, *F. J. Sarrión*, MACB 80303; Fuencaliente, valle por debajo de "El Abuelo", 5-II-1997, *A. R. Burgaz, I. Martínez & F. J. Sarrión*, MACB 80301; Cuenca: Campollos-Sª, Serranía de Cuenca, Valdeliebres, 3-V-1998, *G. Aragón & I. Martínez*, MA-Lichen 10740; Huerta del Marquesado, Sª de Valdemeca, arroyo de la Hoz, 2-V-1998, *G. Aragón & I. Martínez*, MA-Lichen 10200; La Cierva, Serranía de Cuenca, Dehesa de los Palancares, Las Torcas, 14-III-1998, *G. Aragón & I. Martínez*, MA-Lichen 11674, MACB 74638; Las Majadas, Serranía de Cuenca, La Dehesa, 4-I-1998, *G. Aragón & I. Martínez*, MA-Lichen 11044; Nacimiento del río Cuervo, 28-V-1996, *A. R. Burgaz & I. Martínez*, MACB 93807; Serranía de Cuenca, pista que va desde Las Majadas a Uña, El Majadal, 4-I-1998, *G. Aragón & I. Martínez*, MA-Lichen 11640; Sª de Tragacete, nacimiento del río Júcar, 2-V-1998, *G. Aragón & I. Martínez*, MA-Lichen 10251; Valdemeca, Sª de Valdemeca, arroyo Vertiente, 29-V-1996, *A. R. Burgaz & I. Martínez*, MACB 93799; Gerona: Alp, Supermolina, Sª de Cadí, 2-VII-1996, *A. R. Burgaz*, MACB 93808, MACB 93817; Queralbs, Vall de Núria, 18-VIII-2006, *A. R. Burgaz*, MACB 102870, MACB 102871; Setcases, estación de esquí Vallter, 17-VIII-2006, *A. R. Burgaz*, MACB 95398, MACB 102849; Vallfogona del Ripollés, 10-XII-1995, *G. Aragón, A. Herrero & I. Martínez*, MACB 93810; Granada: Huetor, Sª de Huetor, Parq. Nat. de Huetor, Fuente Fría, 20-IV-2006, *A. R. Burgaz*, MACB 102872, Fuente Fría, 20-IV-2006, *A. R. Burgaz*, MACB 102846; Guadalajara: Bustares, Sª de Alto Rey, 23-VIII-2003, *A. R. Burgaz*, MACB 95281; Cantalojas, valle del Sorbe, 8-VIII-1986, *Burgos, Cardiel & Morales*, MACB 20738; Checa, Sª del Tremedal, cerca del cerco del Moro, 5-V-1998, *G. Aragón & I. Martínez*, MA-Lichen 10873; Entre Galve de Sorbe y Condemios, 17-VI-1972, *Bellot, Carballal & Ron*, MACB 5106; Gascueña de Bornoba, Sª de Alto Rey, 23-VIII-2003, *A. R. Burgaz*, MACB 95280; La Fuensaviñán, 12-X-2002, *A. R. Burgaz*, MACB 93805; Orea, Sª del Tremedal, Cerro Caballo, 3-V-1998, *G. Aragón & I. Martínez*, MA-Lichen 10785; Sigüenza, 12-X-2002, *A. R. Burgaz*, MACB 93595; Huesca: Bielsa, valle de Pineta, P. N. de Ordesa y Monte Perdido, 28-VII-1998, *A. R. Burgaz*, MACB 94415; Bielsa, valle de Pineta, río Cinca, 25-VII-2008, *A. R. Burgaz*, MACB 102889; Biescas, Valle de Lasieso, 9-IX-1994, *A. R. Burgaz*, MACB 93616; Biescas, valle del Asiego, 9-IX-1994, *A. R. Burgaz, Martínez & Sarrión*, MACB 64874; Escalona, Urbez, valle del río Vellos, fuente de los suspiros, P. N. de Ordesa y Monte Perdido, 28-VII-1998, *A. R. Burgaz*, MACB 94429; Formigal, Barranco de Brocuso, 6-IX-1991, *T. Ahti & A. R. Burgaz*, MACB 45975; Formigal, Barranco de Brocuso, 6-IX-1991, *T. Ahti & A. R. Burgaz*, MA-Lichen 4037; Hecho, Selva de Oza, 17-VII-1992, *I. Martínez*, MACB 93816; Nocito, Sª de Guara, barranco de Lapillera, 29-VI-1995, *A. Herrero*, MACB 93614; Ordesa, senda hasta cascada del estrecho, 19-V-2002, *J. Etayo*, MA-Lichen 13320; Plan, 4-X-1991, *A. Buades*, MACB 45974; Plan, refugio de Labasar, valle de Gistau, 27-VII-2003, *A. R. Burgaz, Martínez & Sarrión*, MACB 93600; Salinas, valle de Gistau, 27-VII-2003, *A. R. Burgaz*, MACB 93596; Santa Cruz de la Serós, Sª de San Juan de la Peña, monasterio alto de S. Juan de la Peña, 21-VIII-2006, *A. R. Burgaz*, MACB 93780, MACB 95359; Saravillo, hacia refugio Labasar, valle de Gistau, 27-VII-2003, *A. R. Burgaz*, MACB 93599, MACB 93598; Valle de Gistau, 27-VII-2003, *A. R. Burgaz*, MACB 93597; La Coruña: Muros, Playa de

Lariño, 2-IX-2002, *A. R. Burgaz*, MACB 94416; La Rioja: Monterrubio, Sª de la Demanda, 10-IV-1990, *A. R. Burgaz*, MACB 45957; Villoslada de Cameros, Pista del Sillar, 30-III-2005, *S. Pérez-Ortega*, MA-Lichen 15227; León: Cofiñal, pinar de Lillo, 6-VIII-2003, *A. R. Burgaz*, MACB 93813; Puerto de Ancares, 12-IV-1987, *A. R. Burgaz*, MACB 45955; Sena de Luna, 13-VIII-1990, *F. Sarión*, MACB 45967; Tejedo de Ancares, 11-IV-1987, *A. R. Burgaz*, MACB 45954; Lérida: Espot, Subida lago de S. Mauricio, 27-VII-1998, *A. R. Burgaz*, MACB 94430, MACB 94218; La Coma i la Pedra, Tuixén, Sª del Port del Compte, camino de la estación de esquí, 4-VII-1996, *A. R. Burgaz & I. Martínez*, MACB 93804; Las Bordas, valle de Artiga de Lin, 20-VIII-1993, *I. Martínez*, MACB 94417; Montellá. Sª del Cadí, camino al refugio de Prat d'Aguiló, 2-VII-1996, *A. R. Burgaz & I. Martínez*, MACB 94418; Lugo: Cobelas, Castroverde, 31-X-1994, *E. Munin*, MACB 94419; Gundriz, Municipio Samos, valle de Louzara, 24-III-2005, *A. Noguerol Seoane*, MACB 102848; Queixoiro, arroyo invernal, 21-VII-2006, *A. R. Burgaz*, MACB 93781, MACB 102884; S. Martín de Suarna, 21-VII-2006, *A. R. Burgaz*, MACB 95427; Madrid: Montejo de la Sª, 21-VII-2003, *G. Amo & A. R. Burgaz*, MACB 88652; Montejo de la Sª, 22-III-2002, *G. Amo & A. R. Burgaz*, MACB 88648; Montejo de la Sª, 8-III-2002, *P. Aguilar, G. Amo & A. R. Burgaz*, MACB 93620; Rascafria, finca Ontalva, 12-VI-2007, *A. R. Burgaz*, MACB 95199; Navarra: Baraibar, Sª de Aralar, pasado Casa Forestal, alrrededores del Dolmen de Erbilerri, 29-VII-2008, *A. R. Burgaz*, MACB 102857, MACB 102854; Eugui, 7-IX-1991, *T. Ahti & A. R. Burgaz*, MACB 45960; Orbaiceta, Selva de Irati, 7-IX-1991, *T. Ahti & A. R. Burgaz*, MA-Lichen 4038; Orbaiceta, Selva de Irati, 7-IX-1991, *T. Ahti & A. R. Burgaz*, MACB 45961, MACB 45976; Oronoz-Mugaire, 8-IX-1991, *T. Ahti & A. R. Burgaz*, MACB 45962; Pontevedra: Cerca de Lucio, 27-XII-1976, *R. Carballal*, MACB 8525; Isla de Ons, 27-VIII-2002, *A. R. Burgaz*, MACB 94422; La Guardia, Monte Santa Tecla, 29-VIII-2002, *A. R. Burgaz*, MACB 94423, MACB 93818, MACB 93621; Salamanca: El Cabaco, Sª de la Peña de Francia, 6-XI-2005, *A. R. Burgaz*, MACB 102868; Segovia: Riofrío de Riaza, valle del río Riaza, La Cañada, 30-IV-1994, *G. Aragón & I. Martínez*, MA-Lichen 4892; Sevilla: Alanis, Parq. Nat de Sª Norte, 23-IV-2006, *A. R. Burgaz*, MACB 102867; Soria: Laguna Negra, Sª de Urbión, 7-IX-2003, *A. R. Burgaz*, MACB 93593, MACB 93594, MACB 93728, MACB 93815; Muriel Viejo, 12-VIII-2008, *A. R. Burgaz*, MACB 102855, MACB 102856, MACB 102892; Pto. de Santa Ines, vertiente norte, 7-IX-2003, *A. R. Burgaz*, MACB 93803; Santa Ines, 7-IX-2003, *A. R. Burgaz*, MACB 95282; Santa María de las Hoyas, Sª de Nafría, cañón del río Lobos, 9-III-1996, *G. Aragón & I. Martínez*, MA-Lichen 7604; Vinuesa, 7-IX-2003, *A. R. Burgaz*, MACB 93602, MACB 93619, MACB 93820; Teruel: Linares de Mora, Sª de Gúdar, pasado el Puerto de Linares, 15-VI-1996, *G. Aragón & I. Martínez*, MA-Lichen 7549; Mora de Rubielos, Sª de Gúdar, 16-VI-1996, *G. Aragón & I. Martínez*, MA-Lichen 7822; Orihuela del Tremedal, 11-IV-1991, *A. R. Burgaz*, MACB 45959; Orihuela del Tremedal, Sª del Tremedal, el Castillejo, 4-V-1998, *G. Aragón & I. Martínez*, MA-Lichen 10475, MA-Lichen 10797; Puerto de Bronchales, 12-VI-1991, *A. R. Burgaz*, MACB 45970; Toledo: Navamorcuende, ascenso a las Cruces, vertiente sur, 23-III-1996, *S. Vázquez, A. R. Burgaz & M. Acón*, MACB 60292; Zamora: Ribadelago, subida Pico del Fraile, P. Nat. Lago de Sanabria, 9-IX-1998, *A. R. Burgaz, S. Casas & I. Rodríguez de Lope*, MACB 70233; Sotillo de Sanabria, subida arroyo de las Truchas, P. Nat. Lago de Sanabria, 8-IX-1998, *A. R. Burgaz, S. Casas & I. Rodríguez de Lope*, MACB 70234; Zaragoza: Circo de San Miguel, Sª del Moncayo, 13-IX-2003, *A. R. Burgaz*, MACB 93802, MACB 93601; Sª del Moncayo, 13-IX-2003, *A. R. Burgaz*, MACB 93592, MACB 93819; Canary Islands: La Gomera, P. Nat. de Garajonay, " Las Cancelas", 19-II-2002, *D. Sicilia*, MACB 93479; Tenerife: Las Mercedes, Monte de las Mercedes, hacia los Batanes, 16-VI-2007, *A. R. Burgaz*, MACB 102851; Las Mercedes, Monte de las Mercedes, hacia los Batanes, 16-VI-2007, *A. R. Burgaz*, MACB 102853; Las Mercedes, Monte de las Mercedes, mirador del pico del Inglés, 14-VI-2007, *A. R. Burgaz*, MACB 102977; **Sweden**: Bohuslän: Lagelanda parish, 0.5 Km of Vräland, 7-VIII-1960, *R. Santesson*, H; Dalama: Krylbo, 11-IV-1979, *T. Goward*, H; Krylbo, 12-IV-1979, *T. Goward*, H; Krylbo, 12-IV-1979, *T. Goward*, H; Gästrikland: Hedesunda sn, N om Hyttön, hyttruin, mellan flottningsrännan och europaväg 4, 22-IX-2001, *G. Odelvik, 01327*, S L44430; Härjedalen: Tännäs par., The valley of the river Ljusnan, 1km SSE of Ramundbergets Fjällgård, 16-VII-1973, *R. Santesson*, UPS L-139759; Närke: Svennevad, Korsmon, *G. Kjellmert*, H; Öland: Böda parish, 0.5 Km SE of Grankulla, 2-VIII-1962, *R. Santesson*, H; Skane: Svalöv, Skaralid, shady, 7-VI-1985, *R. Skytén*, H; Sverige: Pite lappmark, Arjeplog sn, 4500 m SO om Jäkkvik, kapellet, 21-VIII-2006, *Göran Odelvik*, S F60224; Södermanland, Huddinge sn, Visättra 1950 m. SSO om Flemingsbergs gard, 6-X-2003, *G. Odelvik 03500*, S L60530; Uppland: Danmark par., Linnés Hammarby, 2,4-2,5 Km SE of Damark church, the hill NE of the hauses ("Hammeren"), 15-IX-2007, *G. Thor*, UPS L-167617; Danmark par., Linnés Hammarby, 2,4-2,5 Km SE of Damark church, the hill NE of the hauses ("Hammeren"), 15-IX-2007, *G. Thor*, UPS L-167624; Värland: Ransäter par., Munkfors, liten lodyta i skog vid i Forsviken, 3-IV-1949, *S. Sundell*, UPS L-75554; Uppsala, Fiby nature reserve, 2-VI-1985, *R. Skytén*, H; Västmanland: Arboga, Lindesdal, 8-V-1951, *G. Kjellmert*, MA-Lichen 1157; **Switzerland**: Valais: Wallis Kurhauspad, Evolène, 23-VII-1990, *L. Spier*, L 753075; **Thailand**: Doi Suthep-Pui National Park, 28-III-2005, *S. Parmmen*, H; Phu Him Rougra National Park, 1-V-2005, *S. Paommen*, H; **Turkey**: Trabzon: Akcaabat town, Bozdogan district, V-2005, *K. Yazici*, H; **United States**: Florida: Approximately 6.4 Km southeast of Alexander Springs, 64.4 Km east to southeas of Ocala, 4-I-2002, *R. Rosentreter*, FH; Massachusetts: Berkshire County, Mount Washintong Towship, Mount Everett, 13-X-2000, *P. May*, FH; Worcester County, Lancaster, Devens Reserve Forces Training Area, SE of Clear Pond, west of Bivouac Road. Hemlock-Northern Hardwood Forest, 8-IV-1998, *E. Lay*, FH; Worcester County, Princeton Wachesett Mountain State Reservation, 14-IV-1998, *P. May*, FH; Middlesex, Lincoln, Adams Woods, 4-XI-2001, *B. Huggett*, FH; Massachusetts: Phymouth Co., Hingham, Boston Harbor Island National Park Area, Bumpkin Island, 31-VII-2001, *D. Greene*, FH; New Hampshire: Carroll County, Moultonboro, Ossipee Mountains, Bald Knob Trail, 15-VII-2007, *M. Schmull*, FH 259371; New Jersey: Burlington Co. W of Red Road (Stevenson Road), Oswego River Preserve, 6-IX-2003, *J. C. Lendemer & M. J. Moody*, UPS L-160142; New Jersey: Burlington County, west of Red Road (Stevenson Road), Oswego River Preserve, 6-IX-2003, *C. Lendemer 1206 & M. J. Moody*, S L59663; Washington: Mple mead Farm, 1 mile Toureship, Whatcom, 12-VII-1950, *A. W. C. T. Herre*, L 794577; Near Montesano, Chehalis county, 28-VI-1898, *E. G. Heller*, L 794581; **United Kingdom**: Scothland: Dunberg, Argyll District, Dunstaffnag Castle, 13-VI-2002, *A. R. Burgaz*, MACB 94411.

***Cladonia conista* Robb. ex Evans**

Australia: Victoria: Mirimbach, by side Mt. Stirling Rd., 5-XI-1986, *A. W. Archer,* H; **Chile**: Región XII, Magallanes y Antártica Chilena: Puerto Natales, seno Última Esperanza, 30-I-2005, *A. R. Burgaz,* MACB 92023; **Finland**: Pyluáltó, 12-VI-1947, *L. Fagerstran,* H; Kuusamo: Paljakka, SW of Kiukaakorva rapids, 8-VII-1981, *T. Ahti,* H; North Karelia: Polvijärvi, Sola, Paljakka, 27-IX-2003, *T. Ahti,* H; Päijänne Tavastia: Nastola, Seesta, Kikkegneissikallion päällyställä ohuella kivennäismaalla, niukka, 13-X-2002, *V. Haikonen,* H; South Karelia: Laatokan Karjala, Parikkala, Joensuu, Taplavaara, 13-IX-2003, *T. Ahti,* H; Savitaipale, lyytikkälä, 29-VI-1994, *T. Ahti, F. J. Daniëls, H. Bültmann,* H; Southern Savonia: Koikkala, SE of Mäntymäki, 15-IV-1992, *T. Ahti,* H; Uusimaa: Askola, Kirkonkylä, opposite to junction of road to Pappila, 26-VII-1995, *T. Ahti,* H; Kirkkonummi, 1.5 Km NE of Lillkansokog on Porkkala road, 5-V-2006, *T. Ahti & E. Kuznetsova,* H; Ruotsinpyhtää (Strömfors), Tesjoki, by HighwY E3 at the Loviisa boundary, 23-V-1987, *T. Ahti,* H; Siuntio, Lappers, E of Björnberget, 11-X-1989, *T. Ahti,* H; **Germany**: Bavaria: Angburg, *M. Britzelmayr,* H; North Rhine-Westphalia: Coesfeld, Dülmen, west Münsterland, Trodenrasen, 26-I-1989, *S. Paus,* F C0054634F; **Russia**: Kursk Oblast: By village BuBel, 29-IX-2006, *N. T.*

Zolotuchin, H; **Spain**: Álava: Tertanga, Pto. de Orduña, 25-VII-2006, *A. R. Burgaz,* MACB 97590; Asturias: Felechosa, Foces del Pino, 5-VIII-2003, *A. R. Burgaz,* MACB 95643; Ávila: Peguerinos, campamento de Peñas Blancas, 8-V-2005, *A. R. Burgaz,* MACB 92789; Piedrahita, Pto. de Peñas Negras, 21-XI-2004, *A. R. Burgaz,* MACB 96763; Barcelona: El Brull, P. Nat. del Montseny, coll de Formic, 16-VIII-2006, *A. R. Burgaz,* MACB 95783; Montseny, P. Nat. del Montseny, Turó de l'Home, 16-VIII-2006, *A. R. Burgaz,* MACB 95664; Cáceres: Villar del Pedroso, Garganta del Mesta, 4-I-1995, *G. Aragón & I. Martínez,* MACB 97940; Villar del Pedroso, Sª. del Hospital del Obispo, 4-I-1995, *G. Aragón & I. Martínez,* MACB 61608; Cantabria: Camaleño, Invernales de Mato, 8-VI-1994, *A. R. Burgaz,* MACB 61609; Camaleño, Pido, P. Nac. Picos de Europa, 15-XI-2003, *G. Amo, A. R. Burgaz, I. Martínez & M. G. Otálora,* MACB 89751; Castellón: Chóvar, Sª de Espandán, 11-IV-2003, *A. R. Burgaz,* MACB 97591; Ciudad Real: Villamanrique, estribaciones de Sª Morena, 6-II-2003, *A. R. Burgaz,* MACB 97592; Gerona: Puigcerdá, coll de Tosses, 18-VIII-2006, *A. R. Burgaz,* MACB 97161; Queralbs, Vall de Núria, 18-VIII-2006, *A. R. Burgaz,* MACB 97942; Sant Sadurní d'Osormort, La Selva, 15-IX-2007, *A. R. Burgaz,* MACB 96023; Granada: Lugros, Sª. Nevada, P. Nac. de Sª. Nevada, cabecera del río Alhama, 21-IV-2006, *A. R. Burgaz,* MACB 95670; Guadalajara: Sigüenza, 12-X-2002, *A. R. Burgaz,* MACB 97159; Huesca: Ansó, barranco de las Eras, subida al collado Pinar Alto, 22-VIII-1992, *G. Aragón, A. Herrero & I. Martínez,* MACB 98033; Oza, subida al valle de Aguas Tuertas, valle de Hecho, 23-VII-2000, *A. R. Burgaz,* MACB 92796; La Rioja: Anguiano, 8-IX-2004, *A. R. Burgaz,* MACB 93026; León: Pto. de San Isidro, 5-VIII-2003, *A. R. Burgaz,* MACB 95645; Madrid: Miraflores de la Sª, Pto. de Canencia, 14-II-2000, *A. R. Burgaz & I. Martínez,* MACB 92896; Menorca: Ciutadella, cala Galdana hacia cala Macarella, 29-XI-2007, *A. R. Burgaz,* MACB 98085; Orense: subida Cabeza de Manzaneda, Sª de Queixa, 24-VIII-2002, *A. R. Burgaz,* MACB 98080; Subida a Cabeza de Manzaneda, 24-VIII-2002, *A. R. Burgaz,* MACB 98080; Soria: Lubia, Altos de Lubia, 7-IX-2003, *A. R. Burgaz,* MACB 98041; Tarragona: Conca de Barberá, Prades, 22-V-1998, *A. R. Burgaz,* MACB 92770; Toledo: San Pablo de los Montes, 22-V-2002, *A. R. Burgaz,* MACB 92772; **South Africa**: Cape province: Cape Peninsula, S. End of Constantiaberg, 14-I-1945, *F. M. Leighton*, H; **United States**: Connecticut: Litchfield county, 1/2 mile north of CT Route 126, west od Sand Road, Canaan, 21-IX-2003, *J. C. Lendemer et al.,* H; New Jersey: Gloucester county, Winslow Wildlife Management Area, 1.5 mi W of NJ 322, W of Great Egg Harbor River, 6-II-2009, *J. C. Lendemer et al.,* H; Pensilvania: Blair county, State Game Lands No 73, Tussey Mountain (summit ridge), SE of PA 164 in¡mmediately W of Bedford County line, opposite entrance of Mid State Trail, Woodbury Township, 22-IV-2008, *J. C. Lendemer et al.,* H.

***Cladonia cornuta* (L.) Hoffm.**

Austria: Salzburg: Salzburg, Zillertaler Alpen, Gerlosplatte, 10-IX-1973, *O. Vitikainen*, H; **Canada**: Manitoba: SE of Flin Flon, Thompson Mine, Little Spruce Lake Road, 24-V-2004, *T. Ahti, M. Piercey-Normone & T. Booth*, H; Newfoundland: Island of Newfoundland, Norther Peninsula, Burnt Cape Ecological Preserve, 14-VIII-2007, *J. C. Lendemer & A. Moroz*, H; Nova Scotia: Sable Island, 13-VIII-2007, *H. S. Richardson*, H; **Chile**: Región XII, Magallanes y Antártica Chilena: Puerto Natales, seno Última Esperanza, 30-I-2005, *A. R. Burgaz*, MACB 92203; **Denmark**: Jylland: Silkeborg, Vesterskoven, Kongshus, 5-X-1967, *R. Bäck*, H; **Estonia**: Pölva: Pölvamaa, Värska, Lutepää, Pikkaliiva Sand Field Landscape Reserve, 27-IV-1991, *T. Ahti, H. Trass & T. Randlane*, H; Pölvamaa, Värska, Lutepää, Pikkaliiva Sand Field Landscape Reserve, 27-IV-1991, *T. Ahti, H. Trass & T. Randlane*, H; **Finland**: Päijänne Tavastia: Hämeekoski, Miehola, Mustikkamäki, 22-V-2003, *V. Haikonen*, H; Hausjärvi, Lavinto, Miehonkallio, 15-V-2003, *V. Haikonen*, H; Hollola, Sudenpesänmäki, 9-VIII-2008, *V. Haikonen*, H; Lahti Järvenpää, 6-IX-2008, *V. Haikonen*, H; Lahti, Kujala, Lakkilantie, 4-IX-2008, *V. Haikonen*, H; Nastola, Haarankylä, Kivinenportti, 23-IV-2003, *V. Haikonen*, H; Pirkanmaa: Tampere, Aitolahti, 27-X-2002, *M. Kääntönen*, H; Uusimaa: Artjärvi, Hietana, Litkanmäki, 3-VII-2003, *V. Haikonen*, H; Artjärvi, Hiitelä, Pulikankallio, 9-IV-2003, *V. Haikonen*, H; Hyvinkää, Myllykylä, 21-VIII-1993, *E. Rinne*, H; Lapinjärvi, Koivualhonmäki, 25-VIII-2005, *V. Haikonen*, H; Myrskylä, Humalakoski, 16-IV-2005, *V. Haikonen*, H; Myrskylä, uusisilta, Uudensillankallio, 15-IV-2003, *V. Haikonen*, H; Nurmijärvi, Perttula, Äijänkallio, 29-IX-1995, *T. Ahti, A. R. Burgaz, I. Martínez & O. Vitikainen*, MACB 96300; Orimattila, Järvikylä, Vuorenmäki, 19-XI-2006, *V. Haikonen*, H; Orimattila, Kaitala, Kairesuonkangas, 20-XI-2005, *V. Haikonen*, H; Orimattila, Leitsamaa, Porttikallio, 25-XI-2005, *V. Haikonen*, H; Orimattila, Pennala, Rautamäki, 26-IV-2002, *V. Haikonen*, H; Porvoo rural parish, island Emäsalo, Varlaxudden, 10-X-1995, *T. Ahti, A. R. Burgaz, I. Martínez & O. Vitikainen*, MACB 96301; Pukkila, Haarajoki, Venunmetsä, 8-X-2008, *V. Haikonen*, H; **Germany**: Schwaben: Auf Sandboden im Föhregehülze bei den Schwalbmühlen unweit Wending, VII-1883, *Arnold*, H; **Italy**: Trentino-Alto Adige/Südtirol: Paneveggio und Bellamonte in Südtirol, 6-VIII-1885, *Arnold*, H; **Mongolia**: Lärchenwald bei Ongor-Ulan, 28-VI-1978, *S. Huneck*, H; South Hangay: Hangay Mts., Tarbagatay Range, Dzavham aimak, 7-I-1976, *L. G. Biazrov*, H; **Netherlands**: Friesland: Ameland, Nes, Duinoord, 16-IX-1995, *A. Aptroot*, H; **Norway**: Eastern Norway: Oppland, Sel herred, Rondane, Hövringen, 9-VII-1965, *J. Suominen*, H; Northern Norway: Finnmark, Sör-Varanger, Neiden, NE part of Faerdesmyra, VIII-1965, *R. D. Vorren*, H; Tromso, Kvaenangen, 1.5 Km SW of Kjaekan, 15-VIII-1970, *T. Ahti*, H; **Russia**: Arkhangelsk Oblast: Gouv. Archangelsk, opp. Onega, Andosero, 31-VII-1899, *J. I. Liro & A. K. Cajander*, H; Gub. Archangelsk, Ad flum. Peza, prope Lobanovskaya, 14-VI-1891, *A. O. Kihlman*, H; Gub. Archangelsk, Nyafta, 12-VI-1891, *A. O. Kihlman*, H; Shchuch'e Lake, 21 Km NE of Tsukhcherema on E bank of Severnaya Dviba, 18-VII-1990, *L. Hämet*, H; Karelia Republic: Gouv. Olonets, Archangelskij pogost ad ft. Onega, 24-VIII-1899, *J. I. Liro & A. K. Cajander*, H; Leningrad: Juksovo, ca. 7Km SE of Kiskovshchina by Lake Juksovskoe, on the N side of Lake Vashkus, 9-IV-1942, *J. Sarvela*, H; Sakha Republic: Kangalassy Dist., Natural National Park Lenskie Stolby, south bank of Lena River, 0.5-1 Km S of mouth of Labyiya River, 30-VI-2002, *T. Ahti*, H; **Spain**: La Rioja: Lumbreras, 21-X-1983, *A. R. Burgaz*, H; Soria: Santa Inés, 7-IX-2003, *A. R. Burgaz*, MACB 94344; **Sweden**: Lappland: Torne Lappmark, Jukkasjärvi, Lainio by vid Taanikurkkio, 23-IX-1911, *G.Lang*, H; Torne Lappmark, Karesuando, Luongasjoki-Suija, 10-IX-1911, *G.Lang*, H; Södermanland: St. Malm, Sandviksskogen, 18-VII-1915, *O. Gust & A. Malme*, H; Uppland: Väsby, 30 Km NE of Uppsala, 5-IV-1979, *T. Goward*, H; **Switzerland**: Wallis: Riederalp, Aletschwald NW-slope, 9-IX-1982, *S. Hyvönen*, H; Riederalp, Aletschwald, NW-slope, 29-VIII-1982, *S. Hyvönen*, H; Riederalp, Aletschwald, NW-slope, 29-VIII-1982, *S. Hyvönen*, H; Riederalp, Riederhorn, 19-VIII-1982, *S. Hyvönen*, H; Riederalp, Teiffe Wald, 25-VIII-1982, *S. Hyvönen*, H.

***Cladonia corymbescens* Nyl.**

India: Sikkim: East Sikkim dist., Kupup, north border side (near China), 19-IX-1998, *G. P. Sinha*, H; North Sikkim Dist., Lachen Gumpa, 28-VIII-1999, *G. P. Sinha*, H; West Sikkim Distr., near Bakhim, 13-X-1995, *G. P. Sinha*, H; West-Sikkim Dist., Phithang Dzongri 3 Km track, 12-V-1994, *G. P. Sinha*, H; **Nepal**: Khumbu Rimal, 8-IV-1981, *S. Remus & M. Menzel*, H; Eastern Region: Mechi, Frontière Népal-Sikkim, 2-X-1971, *P. Ozenda*, H; Far-western Region: Seti, Descente de Dahachaur, confluent de la Nhuna Khola, 17-V-1973, *P. Ozenda*, H; **New Guinea**: Mount Wilhelm, 28-II-1965, *A. C. Jermy*, H; **Thailand**: Phu Leaung Wildlife center, Northeasthern Thailand, 29-VIII-2005, *S. Parnmem*, H;

***Cladonia corsicana* (Rondon & Vězda) Pino-Bodas, Burgaz & M. P. Martín**

Material estudiado enumerado en el ARTÍCULO II

***Cladonia ecmocyna* Leighton**

Greenland: Aappilattoq, 23-VI-2004, *E. S. Hansen*, H; Ammassalik, island west of the Sermilik Station, 11-VIII-2001, *E. S. Hansen*, H; Isortoq, 24-VI-1998, *E. S. Hansen*, H; Qeqertaq, 4-VIII-2003, *E. S. Hansen*, H; **Norway**: Østlandet: Telemark, Vinje, Kleivsli, 6-VIII-2000, *S. Rui & E. Timdal*, H; **Spain**: Burgos: Neila, Sª de Neila, Laguna Larga, 6-IX-2003, *A. R. Burgaz*, MACB 101650; Madrid: Rascafría, Sª de Guadarrama, P. Nat. de Peñalara, cumbre Hermana Mayor, 30-V-2006, *A. R. Burgaz*, MACB 101649; **Switzerland**: Wallis: Glestsch, auf dem Glestschboden auf Erde, 25-VIII-1979, *H. Schindler*, H; Riffelboden, Zermatt, 13-IX-1972, *A. M. Burnet*, H; **United States**: Colorado: Boulder Co., 4.8 Km west of Allens Park in Rocky Mountain National Park, Calypso Cascades along Cony Creek, 23-IX-1967, *S. Shushan*, H; Horse Rock Ridge near Mabel, Linn Co., 6-VI-1989, *S. Hammer*, H; Washington: Near entrance to Silver Spring Campground, Mt. Baker Snoqualmie National Forest, 12-VI-989, *S. Hammer*, H.

***Cladonia ecmocyna* subsp. *intermedia* (Robbins) Ahti**

United States: Idaho: Idaho county, near Burgdorf, 25-IV-2007, *M. K. Advaita*, H; Montana: 8 Km SW of East Glacier, 3-VII-1987, *A. DeBolt, B. McCune & R. Rosentreter*, H; Numa Ridge Trail, Bowman Lake Camground, Glacier National Park, Flathead Co., 18-VI-1989, *S. Hammer*, H; Two Medicine Pass, approx. 12 air Km W of East Glacier, 10-VII-1986, *A. DeBolt*, H; New Mexico: Taos County, Gold Hill, 10-VIII-1952, *H. A. Imshaug*, H.

***Cladonia farinacea* (Vainio) Evans**

Argentina: Tierra de Fuego: Ushuaia, Baliza, 19-XII-1969, *H. Roivainen*, H; Ushuaia, Río Olivia, Las Culebras, 15-I-1970, *H. Roivainen*, H; Ushuaia-Lapataia road, 3.5 Km W of Ushuia, 26-XI-1971, *H. Imshaug & K. Ohlsson*, H; **Canada**: Nova Scotia: Colchester Co., Portapique Wilderness Area, N of Montrose, E side of Portapique River, 17-V-2004, *T. Ahti*, H; Sable Island, 3-13-VIII-2007, *D. H. S. Richardson*, H; **Chile**: Región XII, Magallanes y Antártica Chilena: Isla de Navarino, Puerto Williams, valle del río Ukika, camino a Media Luna, 18-I-2005, *A. R. Burgaz*, MACB 92078, MACB 92078; **China**: Sichuan: NW Sichuan, Minshan, Nanping Co. Jiu-Zhai-Go, Zha-Wa-Gou, Lake Chang-Hai, 15-IX-1991, *T. Koponen*, H; **Russia**: Irkutsk Oblast: NW des Baikalsees, Oblast Irkutsk, 15-VIII-1984, *P. Clerc, C. Scheidegger & K. Neigung*, H; Kamchatka: Southern slope of Tolbachil volcano, 11-VIII-2006, *D. Himelbrat & I. Stepachikova*, H; Tuva Republic: South Siberia Mountains, Todzhginskaya Valley, State Reserve "Azar", Toora-Khem Village, Tooro Khem River, 29-VII-995, *T. N. Otnyukova*, H; **United States**: Pensylvania: Lackawanna county, Moosic Mountain, Moosic Mountain Barrens, central Moosic Montains, 2 mi SE of Jessup, e of Olyphant, 4-VII-2008, *J. C. Lendemer*, H; Vermont: Bennington Co., Winhall Township, Green Mountain National Forest near Bondville, 31-V-1995, *S. Hammer*, H,

***Cladonia firma* (Nyl.) Nyl.**

Andorra: Port d'Envalira, 03-VII-1996, *A. R. Burgaz & I. Martínez*, MACB 90646; **Portugal**: Alto Alentejo: P. Nat. São Mamede, alto del pico São Mamede, 30-VIII-1997, *A. R. Burgaz*, MACB 90946; P. Nat. Sao Mamede, subida al pico São Mamede, Valle río Lourenço, 10-I-2004, *A. R. Burgaz*, MACB 90681; Beira Alta: Sª do Caramulo, Alcofra, 29NE68, VIII-1941, *G. da Cunha*, herb. Tavares s.n., LISU. Viseu, 26-I-1996, *A. R. Burgaz & I. Martínez*, MACB 66888; Beira Litoral: Campises, Sª de Sicó, 29-I-1996, *A. R. Burgaz & I. Martínez*, MACB 90679; Ribatejo: Ferreira do Zêzere, 29SND69, 1940, *G. de Barros*, herb. Tavares s.n., LISU. Santarém, 11-II-2001, *P. Pinho*, MACB 80945; **Spain:** Ávila: Navamojada, 10-VI-1992, *A. B. Burgaz* , MACB 90658; Piedrahita, 21-XI-2004, *A. R. Burgaz* , MACB 91612; Ramacastañas, 14-XI-2004, *A. B. Burgaz* , MACB 90657; Badajoz: Campillo de Llerena, Km 41 de Castuera a Valencia de las Torres, 04-IV-1993, *E. Fuertes*, MACB 90659; Burgos: San Martín de Humada, 10-VIII-1998, *A. R. Burgaz*, MACB 91615; Cáceres: Salorino, ribera de los Molinos, 09-I-2004, *A. R. Burgaz*, MACB 90682; Alía, Sª del Hospital del Obispo, estrecho de la Peña, arroyo Jarigüela, 04-I-1995, *G. Aragón & I. Martínez*, MACB 91597, 102948; Castañar de Ibor, Sª de Viejas, 3-I-1995, *G. Aragón & I. Martínez*, MACB 92696; Cádiz: Alcalá de los Gazules, El Picacho, P. Nat. de los Alcornocales, 21-IX-2004, *A. R. Burgaz*, MACB 92994; La Almoraima, Castellar de la Frontera, 5-XI-1992, *A. R. Burgaz*, MACB 92992; Ciudad Real: Albaladejo, Sª del Relumbrar, 05-II-2003, *A. R. Burgaz*, MACB 90661; Almodóvar del Campo, valle de Alcudia, camino de Zarzoso, 03-II-1997, *A. R. Burgaz, I. Martínez & F. J. Sarrión*, MACB 90620; Fuencaliente, 29-I-1990, *A. R. Burgaz, E. Fuertes & F. J. Sarrión*, MACB 37191; Fuencaliente, 17-II-1991, *F. Sarrión*, MACB 46589; Fuencaliente, Aº Robledillo de la Hoyas, 05-II-1997, *A. R. Burgaz, I. Martínez & F. J. Sarrión*, MACB 90663; Villamanrique, Sª Morena, 06-II-2003, *A. R. Burgaz*, MACB 91614; Cuenca: Campillo de Altobuey, 06-IV-2003, *A. R. Burgaz*, MACB 90664; San Clemente, 11-V-2004, *A. R. Burgaz*, MACB 92993; Guadalajara: Almiruete, 07-XII-2001, *E. Ron & T. Ballesteros*, MACB 84692; Gascueña de Bornova, Sª de Alto Rey, 23-VIII-2003, *A. R. Burgaz*, MACB 91619; La Fuensaviñán, 12-X-2002, *A. R. Burgaz*, MACB 90649; Orea, arroyo de Montezuela, 19-V-1996, *G. Aragón & I. Martínez*, MACB 91598; Riofrío del Llano Atienza, 17-VI-1972, *Pastrana & R. Carballal*, MACB 5104; Huelva: Ctra. 433, Los Marines a Fuenteheridos, cortijo Agua Rubia, 28-IV-1993, *A. R. Burgaz*, MACB 90666; Punta Umbría, 14-VIII-1996, *A. R. Burgaz*, MACB 90654; Zalamea la Real, 28-VI-1993, *I. Martínez*, MACB 92990; Huesca: Torla, 10-VII-1989, *A. R. Burgaz*, MACB 90653; Jaén: Chiclana de Segura, embalse de Guadalmina, 15-II-2005, *A. R. Burgaz*, MACB 91609; Génave, 23-III-2002, *A. R. Burgaz*, MACB 90652; Santa Elena, 27-X-2005, *A. R. Burgaz*, MACB 92986; León: Puente de Quinto, El Picón, 24-IV-1998, *A. Terrón*, LEB-4457; Madrid: Alpedrete, 15-IV-1994, *A. R. Burgaz*, MACB 90673; Buitrago de Lozoya, 15-XI-1992, *A. R. Burgaz*, MACB 91618; Buitrago de Lozoya, 26-IX-1989, *A. R. Burgaz*, MACB 37194; Colmenarejo, 11-III-1998, *A. R. Burgaz*, MACB 90672; El Cuadrón, 18-IV-1996, *A. R. Burgaz*, MACB 92988; Galapagar, 04-XII-1989, *A. R. Burgaz*, MACB 37193; Hoyo de Manzanares, 01-XI-1989, *A. R. Burgaz*, MACB 37192; La Cabrera, 4-I-2005, *A. R. Burgaz*, MACB 92666; Las Matas, 19-II-2002, *A. R. Burgaz*, MACB 90651; Manzanares el Real, P. Nat. de La Pedriza, Senda de Quebrantaherraduras, 3-V-2005, *A. R. Burgaz*, MACB 92685; Monte de El Pardo, 9-IV-2004, *A. R. Burgaz*, MACB 90669, MACB 92991; Navalagamella, 17-I-1992, *A. Herrero*, MACB 46588; Puerto de Canencia, 30-IX-1999, *A. R. Burgaz*, MACB 90671; Redueña, 11-V-1998, *A. R. Burgaz*, MACB 90668; Torrelaguna, Cotos de Monterrey, 17-I-1997, *A. R. Burgaz*, MACB 90667; Salamanca: Beleña, 5-XI-2005, *A. R. Burgaz*, MACB 91680; Frades de la Sª, Sª de Frades, 5-XI-2005, *A. R. Burgaz*, MACB 91674; Fuenterroble de Salvatierra, Sª de Frades, 5-XI-2005, *A. R. Burgaz*, MACB 91693; Fuentes de Oñoro, 20-XI-1990, *A. R. Burgaz*, MACB 46587; La Alberca, Sª de la Mestas, valle del río Batuecas, 6-XI-2005, *A. R. Burgaz*, MACB 91679;

Las Mestas, Sª de las Mestas, valle del río Batuecas, 6-XI-2005, *A. R. Burgaz*, MACB 91677; Valdemierque, 5-XI-2005, *A. R. Burgaz*; Segovia: Cedillo de la Torre, 23-IV-2001, *A. R. Burgaz & P. Pinho*, MACB 92989; Sevilla: Alanis, P. Nat. de Sª Norte, 23-IV-2006, *A. R. Burgaz*, MACB 92694; Cazalla de la Sª, P. Nat. de Sª Norte, Finca UPA, 23-IV-2006, *A. R. Burgaz*, MACB 92695; Puebla del Río, 21-II-1993, *A. R. Burgaz*, MACB 91616; Soria: Alto de Villaciervos, La Fragua, 31-V-1999, *A. R. Burgaz, M. A. Carrasco & E. Fuertes*, MACB 90650; Lubia, Altos de Lubia, 07-IX-2003, *A. R. Burgaz*, MACB 90670; Villaciervos, 20-IV-1993, *A. R. Burgaz*, MACB 91613; Villaciervos, 10-IX-1991, *A. R. Burgaz*, MACB 46590; Toledo: Belvís de la Jara, 26-XII-2004, *R. Pino-Bodas*, MACB 90683; Consuegra, Sª de Valdehierro, 05-XI-2004, *A. R. Burgaz*, MACB 90655; La Iglesuela, 23-III-1996, *A. R. Burgaz*, MACB 92987; Sevilleja de la Jara, 7-V-2005, *R. Pino*, MACB 91594; Urda, Sª Morrones, Finca El Convento, 05-XI-2004, *A. R. Burgaz*, MACB 90648; Zamora: Almaraz de Duero-Zamora, km 10, 07-IX-1998, *A. R. Burgaz, S. Casas & I. Rodríguez de Lope,* MACB 70169; La Tabla, 06-X-1997, *G. Aragón, A. R. Burgaz & A. Terrón*, MACB 70170.

***Cladonia foliacea* (Huds.) Willd.**

Austria: Mähren: Rokytnatal, 1919, *H. Suza*, H; **Finland**: Regio Aboensis: Korpo, Jurmo, 4-VII-1988, *R. Skytén*, H; V. Dragsfjärd: Tunnhamn, Äspkär, 9-VI-1994, H; Al. Fölglö: Björkör, Norra Askskär Bergsstrak in N S-riktning, 22-VIII-1974, *R. Skytén*, H; **France**: Gard: Remoulins, Sernhac, 23-III-1968, *M. Luotamo & P. Uotila*, H; Meuse, à Foisches, au Sud de Givet, 28-IV-1968, *F. Onraedt*, H; Ille-et-Vilaine: Cossinade, commune de St-Thurial., 4-III-1962, *J. Lambinon*, H; Isère: La Chalp-en-Valjouffrey, 11-X-1977, *Y. Rondon*, H; Languedoc-Rousillon: St. Marsal, Serrat de la Fustera, 12-IV-2007, *V. Haikonen*, H; Seine-et-Marne: 4Km NW of Provins (2 Km S of Montery), 30-V-1977, *T. Ahti*, H; Forêt de Fontaine-bleau, 8-VI-1977, *T. Ahti & J. C. Boissière*, H; Var: One mile east of Vidauban on route N-7, 23-IV-1964, *H. A. Imshuag*, H; Frankrijk Dèp Gironde 3KM o van Lacanau-Ocèan tessen le Huga en Carcans-Plage-Brandgag in pinusbos, op zand, 23-VII-971, *R. Ros*, H; **Germany**: Baden-Wurtemberg: Baden, 1899, *Lanh* , H; Berlin: West Berlin, Heiligensee, Baumberge, 17-V-1969, *T. Ahti*, H; Hessen: Düne bei Bichenbech, 23-VI-1951, *L. Henfsen*, H; Sachsen-Anhalt., 18, *Krause*, H; **Italia**: Calabria: Lago Angitola, 18-I-1986, *D. Puntillo*, H; Toscana: Firenze,Prato, 5 Km N of Prato, monte Ferrato, 25-V-1977, *J. Suominen*, H; Biella: Riserva Naturale della Bessa Cerriore, *D. Isocrono*, H; Grosseto: Castiglione della Pescáia, 10-II-2000, *R. Benesperi*, H; Sardinia: Cagliari, Capoterra (SW of Caglari), Santa Barbara, XII-1987, *F. Turmo & M. C. Loi*, H; 1806, *S.E. Bridel*, H; **Portugal**: Algarve: Aljezur, Praia do Monte Clérigo, 9-XII-2006, *A. R. Burgaz*, MACB 102931; Bensafrim, Sª do Espinhaco de Çao, 9-XII-2006, *A. R. Burgaz*, MACB 102929; Maria Vinagre, hacia la Bahía dos Tiros, 9-XII-2006, *A. R. Burgaz*, MACB 102930; Monchique, Sª de Monchique, Barranco do Banho, 8-XII-2006, *A. R. Burgaz*, MACB 102928; Alto Alentejo: Bencatel, Sª de Ossa, 10-XII-2006, *A. R. Burgaz*, MACB 102932; Evoramonte, 10-I-2004, *A. R. Burgaz*, MACB 90505; Montinho, P. Nat. Sao Mamede, 11-I-2004, *A. R. Burgaz*, MACB 90504; P. Nat. São Mamede, subida al pico Sao Mamede, 10-I-2004, *A. R. Burgaz*, MACB 90501; P. Nat. São Mamede, subida al pico São Mamede, valle río Lourenço, 10-I-2004, *A. R. Burgaz*, MACB 90503; Pégoes, Monte das Piçarras, 09-I-2004, *A. R. Burgaz*, MACB 90500; Baixo Alentejo: Cavalheiro, cabo Sardão, 9-XII-2006, *A. R. Burgaz*, MACB 102935, 102933; Beira Alta: Viseu, San Miguel, 26-I-1996, *A. R. Burgaz & I. Martínez*, MACB 66864; Beira Litoral: Rabaçal, Sª de Sicó, 28-I-1996, *A. R. Burgaz & I. Martínez*, MACB 66859; Aveiro, Costa Nova, 03-IV-1998, *T. Ahti & A. R. Burgaz*, MACB 66863; Estremadura: Sª Arrabida, 8-VIII-2003, *A. R. Burgaz*, MACB 90517; Sª de Sintra, Monasterio de Peninha, 10-I-2004, *A. R. Burgaz*, MACB 91702; Trás Os Montes: Alimonde, Sª de Alimonde, 6-IX-2006, *R. Pino-Bodas*, MACB 102919; Izeda, valle del río Sabor, 5-IX-2006, *R. Pino-Bodas*, MACB 102918; Montezinho, Sª de Montezinho, 8-IX-2006, *R. Pino-Bodas*, MACB 102916, MACB 102911, MACB 102910, MACB 102909; Rebordãos, Sª da Nogueira, 6-IX-2006, *R. Pino-Bodas*, MACB 102917; Rebordãos, Sª da Nogueira, 6-IX-2006, *A. R. Burgaz*, MACB 102943, 102942; Rebordelo, 12-III-1994, *A. R. Burgaz*, MACB 66860; **Spain**: Álava: Puerto de Herrera, 27-IX-1999, *A. R. Burgaz & Rodriguez de Lope*, MACB 91685; Albacete: Bienservida, Sª Alcaraz, 29-III-1993, *A. R. Burgaz*, MACB 90606; Cancarix, 11-V-2004, *A. R. Burgaz*, MACB 90567; Villapalacios, Sª del Relumbrar, 05-II-2003, *A. R. Burgaz*, MACB 90376; Alicante: Alcoy, Sª de Menachaor, Font Roja, 27-V-1996, *A. R. Burgaz & I. Martínez*, MACB 91689; Cabo la Nao, Javea, *Mª J. Cano*, GDA 3087; Carretera de Jalón a Berria, *Mª J. Cano*, GDA 3076; Castalla, Sª de Castalla, Xorret del Catí, 13-V-2004, *A. R. Burgaz*, MACB 90434; Orihuela, Sª de Orihuela, *Mª J. Cano*, GDA 3183; Puerto de Benifallin (Benifallín), *Mª J. Cano*, GDA 3141; Sª de Aril, Barranco de les Planets (Onil), *Mª J. Cano*, GDA 3091; Sª de Aril, Barranco de les Planets, Onil, *Mª J. Cano*, GDA 3092; Sª de la Peña Rubia, peñón de la Rubia (Villena), *Mª J. Cano*, GDA 3090; Sª de Salinas, *Mª J. Cano*, GDA 3045; Sª de Salinas, Loma Cabrera (Villena), *Mª J. Cano*, GDA 3040; Sª de Salinas, lomas del Barranco Barelat, Villena, *Mª J. Cano*, GDA 3046; Sª del Recando, proximo al repetidor de TV, *Mª J. Cano*, GDA 3021; Sª del Traile, proximo a casa de la Botija, Bihar, *Mª J. Cano*, GDA 3016; Sª de Fontanella, loma Rosa, Biar, *M. J. Cano*, GDA 3026; Sª de la Carrasqueta, Jijona, *M. J. Cano*, GDA 3008; Sª del Cid, Alto de la silla del Cid (Petrel), *Mª J. Cano*, GDA 3059; Sª de Salina, barranco de los pozos, *M. J. Cano*, GDA 3032; Almeria: Aulago, Sª Filabres, 7-X-2002, *A. R. Burgaz*, MACB 90644; Cabo de Gata, camino del Cerro del Jayain, *M. Casares*, GDA 3127; Cabo de Gata, Sª de Cabo de Gata, 09-X-2002, *A. R. Burgaz*, MACB 90410; El Cerrón, 18-III-1988, *M. Casares*, GDA 1156, GDA 1163; Loma de los Yesos, 15-IV-89, *M. Casares*, GDA 1561, GDA 1562; Níjar, El Barranquete, 15-XII-1990, *M. J. M. Lirola*, GDA 897; Rioja, 2-II-1990, *M. Casares*, GDA 1974; Rodalquilar, Sª de Cabo de Gata, 09-X-2002, *A. R. Burgaz*, MACB 90425, MACB 90645; Sª Cabo de Gata, Punta de la Polaca entre Hortidruelas y Rodelquilau, *A. R. Burgaz*, MACB 91686; Sª Cabrera, *M. Casares*, GDA 3118; Sª Cabrera, base de mezquita, *M. Casares*, GDA 3111; Sª Filabres, *M. Casares*, GDA 3108; Sª Filabres, puerto de la Virgen, ramblas de la Huerta de la Virgen, *M. Casares*, GDA 3107; Sorbas a 2 Km, 14-IV-1993, *A. R. Burgaz*, MACB 90435; Venta de los Yesos, 19-II-1988, *M. Casares*, GDA 965; Asturias: Llamardal, subida Puerto de Somiedo, 08-VIII-2003, *A. R. Burgaz*, MACB 91696; Ávila: Alto de los Leones, MACB 92689; Navacepedilla de Corneja, 21-XI-2004, *A. R. Burgaz*, MACB 90382; Navamojada, 10-VI-1992, *A. R. Burgaz*, MACB 90384; Ojos-Albos, 5-XI-2005, *A. R. Burgaz*, MACB 91639; Peguerinos, alrededor del campamento "Peñas Blancas", 10-V-1998, *A. R. Burgaz*, MACB 90380; Peguerinos, alrededor del campamento "Peñas Blancas", 8-V-2005, *A. R. Burgaz*, MACB 92661; Piedrahita, 21-XI-2004, *A. R. Burgaz*, MACB 90379; Puerto de Villatoro, 21-XI-2004, *A. R. Burgaz*, MACB 90378; Ramacastañas, 14-XI-2004, *A. R. Burgaz*, MACB 90377; Badajoz: Castuera, 27-III-1991, *A. R. Burgaz & E. Fuertes*, MACB 41481; Barcelona: El Bages, Castellfollit del Boix, Sª de Rubio, 20-V-1998, *A. R. Burgaz*, MACB 90437; L' Anoia, Carme, camino Les Esplugues, 20-V-1998, *A. R. Burgaz*, MACB 90385; El Brull, Parc Natural del Montseny, coll de Formic, 16-VIII-2006, *A. R. Burgaz*, MACB 102927; Montseny, Parc Natural del Montseny, Turó de l'Home, 16-VIII-2006, *A. R. Burgaz*, MACB 102924; Burgos: Atapuerca, 27-IX-1999, *A. R. Burgaz & I. Rodriguez de Lope*, MACB 90416; Ayoluengo, "Páramo de la Lora", 23-VII-1999, *A. R. Burgaz*, MACB 90429; Cuestaedo, subida a las antenas, 25-VII-2006, *A. R. Burgaz*, MACB 102925; Covarrubias, Sª de Covarrubias, 06-IX-2003, *A. R. Burgaz*, MACB 90386; Pesquera de Ebro, 08-VI-1988, *A. R. Burgaz & E. Fuertes*, MACB 41498; Pesquera de Ebro, 24-VII-1999, *A. R. Burgaz*, MACB 90413; San Martín de Humada, 10-VIII-1998, *A. R. Burgaz*, MACB 90387; Sargentes de Lora, 09-VI-1988, *A. R. Burgaz*, MACB 45686; Burgos: Villalaín, 20-

VIII-1998, *A. R. Burgaz*, MACB 90441; Cáceres: Alía, Sª del Hospital del Obispo, estreño de la peña, arroyo Jaríguela, 4-I-1995, *Aragón & Martinez*, MACB 91600; Casas de Miravete, Pto. de Miravete, 09-I-2004, *A. R. Burgaz*, MACB 91694; Castañar de Iboz, Sª de Viejas, 3-I-1995, *Aragón & Martinez*, MACB 91603; Hoyos, Sª de Santa Olalla, 06-I-1996, *A. R. Burgaz*, MACB 90479; Las Mestas, Sª de las Mestas, valle del río Batuecas, 6-XI-2005, *A. R. Burgaz*, MACB 91654; Roblellano, Sª del Cazabal, 3-I-1995, *Aragón & Martinez*, MACB 91605; Salorino, rivera de los Molinos, 09-I-2004, *A. R. Burgaz*, MACB 90419; Cádiz: Alcalá de los Gazules, El Picacho, P. Nat. de los Alcornocales, 21-IX-2004, *Leg: A. R. Burgaz*, MACB 90442, MACB 90388; Barbate, El Soto, 25-V-1997, *A. R. Burgaz, N. Marcos*, MACB 91695, MACB 91688; Castellar de la Frontera, 04-IV-1991, *Sarrión*, MACB 45691; Chiclana de la Frontera, 13-X-1994, *A. R. Burgaz*, MACB 90420; El Soto, paleodunas costeras, 25-V-1997, *A. R. Burgaz*, MACB 90389; Pinar de la Bruña, Barbate, 13-XI-1994, *A. R. Burgaz*, MACB 91608; Vejer de la Frontera, 12-X-1995, *A. R. Burgaz*, MACB 90493; Cantabria: Vega de Liébana, Valneo, 06-VI-1994, *A. R. Burgaz*, MACB 90511; Castellón: Artana, Sª de Espadán, 11-IV-2003, *A. R. Burgaz*, MACB 90482; Chovar, 11-IV-2003, *A. R. Burgaz*, MACB 90483; Convento de Benifasar, P. Nat. Els Ports, 09-IV-2004, *A. R. Burgaz*, MACB 90480; Castellón: Fredes, colonia Europa II, P. Nat. Els Ports, 09-IV-2003, *A. R. Burgaz*, MACB 90481; Ciudad Real: Albaladejo, 05-II-2003, *A. R. Burgaz*, MACB 90392; Almodovar del Campo, valle de Alcudia, camino del Zarzoso, 03-II-1997, *A. R. Burgaz*, MACB 90390; Fuencaliente, 04-I-1990, *Sarrión*, MACB 37184; Fuencaliente, 05-II-1997, *A. R. Burgaz*, MACB 90416; Fuencaliente, 19-I-1990, *A. R. Burgaz & Sarrión*, MACB 37185; Puebla de Don Rodrigo, 16-VI-1993, *A. R. Burgaz*, MACB 90402; Saceruela, Sª de Canalizos, 24-IX-2004, *A. R. Burgaz*, MACB 90391; Villamanrique, Sª Morena, 06-II-2003, *A. R. Burgaz*, MACB 90397, MACB 90484; Villarrubia de los Ojos, Sª de la Cueva, Pº de los Santos, 05-XI-2004, *A. R. Burgaz*, MACB 90396; Viso del Marqués, Sª de San Andrés, Fresnedas Altas, 6-XII-2006, *A. R. Burgaz*, MACB 102938; Córdoba: Fuente Obejuna, Valdeinfierno, 23-IV-2006, *A. R. Burgaz*, MACB 92673; Luque, 08-VIII-2002, *A. R. Burgaz*, MACB 90485; Ventorros de San Jose, 01-IV-1996, *A. R. Burgaz*, MACB 90431; Villaharta, fuente de la Lastrilla, 24-IX-2004, *A. R. Burgaz*, MACB 91703, MACB 90632; Cuenca: Almodovar del Pinar, II-2000, *M. P. James*, MACB 92509; Beteta, Solan de Cabras, Hoz del rio Cuervo, 8-VII-1994, *G. Aragón, A. Herrero, I. Martínez*, MACB 90428; Campillo de Altobuey, 06-IV-2004, *A. R. Burgaz*, MACB 90528; Campillos de Altobuey, 06-IV-1993, *A. R. Burgaz*, MACB 90490; Campillos-Sª, Valdeliebres, 29-V-1996, *A. R. Burgaz*, MACB 90487; San Clemente, 11-V-2004, *A. R. Burgaz*, MACB 90489, MACB 90527; Tragacete, Sª de Tragacete, nacimiento del rio Jucar, 29-V-1996, *A. R. Burgaz & I. Martínez*, MACB 90486; Uña, Ciudad Encantada, 14-IV-1990, *A. R. Burgaz*, MACB 37190, MACB 37198; Valdemeca. Sª de Valdemeca, arroyo Vertiente, 29-V-1996, *A. R. Burgaz*, MACB 90530; Gerona: L' Estartit, Torrella de Montgri, 04-II-1998, *A. R. Burgaz*, MACB 90531; Rosas, 4-II-1998, *A. R. Burgaz*, MACB 91604; San Martín de Ogassa, mirador coll de la Torre, 17-VIII-2006, *A. R. Burgaz*, MACB 102920; Granada: Alfacar, Sª de Huetor, Parq. Nat. de Huetor, La Alfaguara, 20-IV-2006, *A. R. Burgaz*, MACB 92658, MACB 92668; Caniles, Sª Baza, 12-V-2004, *A. R. Burgaz*, MACB 90505, MACB 90407; Entre Cúllar y Baza, 25-III-1988, *M. Casares*, GDA 1611; Loja, Sª de Loja, 17-II-2005, *A. R. Burgaz*, MACB 91706; Loja, Sª de Loja, 17-XI-2005, *A. R. Burgaz*, MACB 91690; Loja, Sª de Loja, 22-IV-2006, *A. R. Burgaz*, MACB 92656; Sª de Baza P. Nat. Sª de Baza, 12-V-2004, *A. R. Burgaz*, MACB 90565; Alcolea del Pinar, 03-XI-1971, *R. Carballal*, MACB 5101; Atienza, 19-V-1971, *R. Carballal*, MACB 8586; Brihuega, 18-I-1970, *R. Carballal*, MACB 5103; Guadalajara: Bustares, Alto de los Teléfonos, Sª de Alto Rey, 23-VIII-2003, *A. R. Burgaz*, MACB 90534; Cantalojas, P. Nat. Tejera Negra, 23-VIII-2003, *A. R. Burgaz*, MACB 90550; Cantalojas, valle del Sorbe Bco. de la Tejera Negra, 20-VI-1985, *J. Burgos & J. M. Cardiel*, MACB 20740; Cardoso, *A. R. Burgaz*, MACB 90535; Fuencemillán, 4-V-2005, *A. R. Burgaz*, MACB 92684; Gascueña de Bornoba, 23-VIII-2003, *A. R. Burgaz*, MACB 91699; Jadraque, 14-IV-2006, *R. Pino-Bodas*, MACB 92660; La Cabrera, 20-IV-1991, *A. R. Burgaz*, MACB 41491; La Fuensaviñán, 12-IX-2002, *A. R. Burgaz*, MACB 90491; Luzón, 11-VI-1991, *A. R. Burgaz & E. Fuertes*, MACB 41489; Monte Alcarria, 11-IV-1971, *R. Carballal*, MACB 5100; Monte Alcarria, 11-VI-1970, *F. Bellot*, MACB 27028; Monte Aldovera, 25-IV-1970, *R. Carballal*, MACB 5102; Monte Aldovera, 28-II-1971, *R. Carballal*, MACB 8535; Negredo, 21-IV-1991, *A. R. Burgaz*, MACB 45089; Pelegrina, 20-IV-1991, *A. R. Burgaz*, MACB 41490; Rebollosa de Jadraque, 19-V-1971, *R. Carballal*, MACB 5099; Sigüenza, 12-X-2002, *A. R. Burgaz*, MACB 90536; Sigüenza, 12-X-2002, *A. R. Burgaz*, MACB 91687; Tamajón, 10-VI-2005, *R. Pino-Bodas*, MACB 91602; Torija, 07-III-1996, *A. R. Burgaz*, MACB 90607; Zaorejas, 02-VI-2003, *A. R. Burgaz*, MACB 90553; Huelva: Almonte, Matalascañas, 29-IV-1993, *I. Martínez*, MACB 90432; Castaño del Robledo, 27-IV-1993, *I. Martínez*, MACB 90433; Encinasola, I-1999, *M. P. Jones*, MACB 92511; Huelva: Matalascañas, 11-VIII-1996, *A. R. Burgaz*, MACB 91701; Huelva: Punta Umbria, 14-VIII-1996, *A. R. Burgaz*, MACB 90554; Zalamea la Real, 28-IV-1993, *I. Martínez*, MACB 90422; Huesca: Arguis, Parque de Guara, Sª de la Gabardiella, antenas de TVE, 22-VII-2006, *A. R. Burgaz*, MACB 102921; Escalona, Urbez, valle del rio Vellos P. N. de Ordesa y Monte Perdido, 28-VII-/1998, *A. R. Burgaz*, MACB 91683; Torla, Valle de Bujaruelo, 10-VII-1998, *A. R. Burgaz*, MACB 90608; Jaén: Albanchez de Mágina, puerto Albanchez, Sª de Mágina, 6-XII-2006, *A. R. Burgaz*, MACB 102940; Albanchez de Mágina, puerto Albanchez, Sª de Mágina, 6-XII-2006, *A. R. Burgaz*, MACB 102939; Barranco de Valdeazores, P. Nat. de Despeñaperros, 9-V-2005, *A. R. Burgaz*, MACB 92688; Chiclana de Segura, 15-II-2005, *A. R. Burgaz*, MACB 90408; Despeñaperros, arroyo Valdeazores, nov-87, *F. J. Sarrión Torres*, MACB 90495; Génave, 27-III-2002, *A. R. Burgaz*, MACB 90555; Mirador, Las Celadillas, 13-III-2003, *A. R. Burgaz*, MACB 90612; Montizón cauce del rio Dañador, 06-II-2003, *A. R. Burgaz*, MACB 90611, MACB 90556; Santa Elena, Sª Morena, 23-IV-2006, *A. R. Burgaz*, MACB 92672; Segura de la Sª, Rio Madera, 22-II-2002, *A. R. Burgaz*, MACB 90494; La Coruña: Muros, Playa de Lariño, 02-IX-2002, *A. R. Burgaz*, MACB 90414; La Rioja: Mansilla, 19-IX-1990, *A. R. Burgaz & E. Fuertes*, MACB 41488; Montenegro de Cameros, 05-VI-2003, *A. R. Burgaz*, MACB 90558; León: Brazuelo, Montes de León, 20-VII-2006, *A. R. Burgaz*, MACB 102922; Isoba, Reserva Nacional de Mampodre, puerto de San Isidro, laguna de Isoba, 22-VII-2006, *A. R. Burgaz*, MACB 102922; Manzanal del Puerto, 03-IX-2002, *A. R. Burgaz*, MACB 90421; Salientes, 28-III-2002, *R. Garcia*, MACB 90557; Lérida: La Noguera, 19-V-1998, *A. R. Burgaz*, MACB 90609; La Vansa-Fornols, Ges, Sª del Cadí, 03-VII-1996, *A. R. Burgaz*, MACB 90613; Arestui, riu Noguera-Pallaresa, 20-VII-2006, *A. R. Burgaz*, MACB 102941; Madrid: Arganda, Dehesa de Arganda, 31-I-1982, *A. R. Burgaz*, MACB 37196; Buitrago de Lozoya, 26-XI-1989, *A. R. Burgaz*, MACB 37187; Canencia, Puerto de Canencia, 13-IX-1998, *A. R. Burgaz*, MACB 70279; Cervera de Buitrago, 19-X-9228, *A. R. Burgaz*, MACB 41486; Ciempozuelos, 17-IV-1991, *A. R. Burgaz*, MACB 41497; Ciempozuelos, 20-XI-1998, *A. R. Burgaz*, MACB 70290; Colmenar de Oreja, finca Encomienda Mayor de Castilla, 15-XI-1996, *A. R. Burgaz*, MACB 90615; Ctra. desde Rascafria al Puerto Morcuera, Km 17-18, arrollo de Santa Ana, 04-IV-1993, *I. Martínez*, MACB ; El Cuadrón, 21-VI-1997, *A. R. Burgaz & S. Casas*, MACB 75222; Embalse de Riosequillo, 21-III-1997, *A. R. Burgaz & S. Casas*, MACB 75234; Galapagar, 04-VII-1989, *A. R. Burgaz*, MACB 37186; Hoyo de Manzanares, 01-XI-1989, *A. R. Burgaz*, MACB 37183; La Cabrera, 4-I-2005, *A. R. Burgaz*, MACB 92662; Las Matas, 19-II-2002, *A. R. Burgaz*, MACB 90498; Manzanares del Real, La Pedriza, 17-XII-1989, *A. Izuzquiza, F. Izuzquiza & A. Mata*, MACB 37182; Miraflores de la Sª, subida al Pto. de Canencia, 08-IV-1996, *A. R. Burgaz*, MACB 90616; Puerto de Canencia, 27-X-2006, *A. R. Burgaz*, MACB 102914; Monte de El Pardo, 09-IV-2004, *A. R. Burgaz*, MACB 90560; Morata de Tajuña, 17-VI-1991, *A. R. Burgaz*, MACB 414999; Navalagamella arroyo de Valquemado, 11-VI-1993, *Aragón & Martinez*, MACB 90559; Ontígola, 9-X-90, *M. Casares*, GDA 2003; Puerto de Canencia, 14-X-1995, *A. R. Burgaz*, MACB 90497; Redueña, 10-V-1998, *A. R. Burgaz*, MACB 90561, MACB 90617; San Juan, Titulcia,

13-X-2002, *A. R. Burgaz*, MACB 91682; San Martín de la Vega, Ctra. a Morata de Tajuña, 10-VI-1993, *G. Aragón, J. Castillo & I. Martínez*, MACB 90436; Torrelaguna, 21-III-1997, *A. R. Burgaz & S. Casas*, MACB 75246; Málaga: Archidona, Hoz de Arroyo Morón, 25-X-2005, *A. R. Burgaz*, MACB 91709, MACB 91709; Ronda, 01-II-1998, MACB 70270; Tolox, Sª de las Nieves, 12-IV-1992, *A. R. Burgaz*, MACB 90619; Murcia: Alhama de Murcia, P. Nat. Sª Espuña, 11-V-2004, *A. R. Burgaz*, MACB 90515; Cartegana, I-2000, *Maurice Pugh James*, MACB 92510; El Berro, P. Nat. Sª Espuña, Barrancos de Gebar, 14-V-2004, *A. R. Burgaz*, MACB 90566; Moratalla, Cañada de la Cruz, base de Santa de Taibilla, 13-V-1993, *I. Martinez*, MACB 90599; Portman, 28-VII-2005, *A. R. Burgaz*, ; Totana, P. Nat. Sª Espuña antenas militares, 14-V-2004, *A. R. Burgaz*, MACB 90620; Zaén de Arriba, 10-IV-2001, *A. R. Burgaz*, MACB 90518; Navarra: Foz de Arbayún, 01-XI-2003, *A. R. Burgaz*, MACB 91684; Navarra: Olazagutia, Sª de Urbasa, 27-IX-1999, *A. R. Burgaz & I. Rodriguez de Lope*, MACB 90499; Palencia: Areños, proximo Pto. De Piedras Luengas, 12-IV-1991, *A. R. Burgaz & N. Marcos*, MACB 41482; Valcovero, Sª de Otero, 18-VII-2004, *A. R. Burgaz*, MACB 90563; Villaviudas, 24-VIII-1983, *A. R. Burgaz*, MACB 37197; Villaviudas, 28-VI-1983, *A. R. Burgaz*, MACB 13086; Salamanca: Beleña, 5-XI-2005, *A. R. Burgaz*, MACB 91641; El Cabaco. Sª de la Peña de Francia, 6-XI-2005, *A. R. Burgaz*, MACB 91648; Frades de la Sª, Sª de Frades, 5-XI-2005, *A. R. Burgaz*, MACB 91645; Frades de la Sª, Sª de Frades, 5-XI-2005, *A. R. Burgaz*, MACB 91636; Fuentes de Oñoro, 23-II-1990, *A. R. Burgaz & E. Fuertes*, MACB 41483; Horcajo de Montemayor, 26-IX-1991, *A. R. Burgaz*, MACB 45692; La Alberca, Sª de la Mestas, Pº del Portillo, 6-XI-2005, *A. R. Burgaz*, MACB 91655, MACB 91708; Las Mestas, Sª de las Mestas, valle del río Batuecas, 6-XI-2005, *A. R. Burgaz*, MACB 91654; Monsagro, Sª de la Peña de Francia, Paso de los Lobos, 6-XI-2005, *A. R. Burgaz*, MACB 91653; Peña de Francia, 26-IX-1991, *A. R. Burgaz*, MACB 45693; Puentes del Alagón, 26-IX-1991, *M. Casares & L. Gutierrez*, GDA 2680; Valdemierque, 5-XI-2005, *A. R. Burgaz*, MACB 91640; Segovia: Aguilafuente, 14-XI-1993, *A. R. Burgaz*, MACB 91704; Arcones-Prádena, 13-IV-2006, *R. Pino-Bodas*, MACB 92657; Cedillo de la Torre, 30-IV-1984, *M. Ventureira*, MACB 14183; Coca, Finca El Sesquero, 20-VII-2006, *A. R. Burgaz*, MACB 102913; Cedillo de la Torre, 28-II-1985, *A. R. Burgaz & Ventureira*, MACB 37195; Puerto de la Quesera, 2-VI-1995, *A. R. Burgaz*, MACB 91601; Sevilla: Alanis, Parq. Nat. de Sª Norte, Mirador Loma del Aire, 23-IV-2006, *A. R. Burgaz*, MACB 92674; Aznalcazar, 7-XII-2006, *A. R. Burgaz*, MACB 102934; Cazalla de la Sª, Parq. Nat. de la Sª Norte, 23-IV-2006, *A. R. Burgaz*, MACB 92671; Cazalla de la Sª, Parq. Nat. de la Sª Norte, UPA, 23-IV-2006, *A. R. Burgaz*, MACB 92670; Cazalla de la Sª, Sª de la Grana, Parq. Nat. de la Sª Norte, 23-IV-2006, *A. R. Burgaz*, MACB 92669; Puebla del Rio, 21-II-1993, *Herrero*, MACB 90507; Puebla del Río, cañada de los Pájaros, 7-XII-2006, *A. R. Burgaz*, MACB 102937, 102936; Soria: Almazán, 06-IV-1993, *A. R. Burgaz*, MACB 90570; Alto de Villaciervos, La Fragua, 31-V-1999, *A. R. Burgaz, M. A. Carrasco & E. Fuertes*, MACB 90520; Altos de Villaciervos, 21-IX-1990, *A. R. Burgaz & E. Fuertes*, MACB 41496; Berlanga de Duero, 13-IV-2006, *R. Pino-Bodas*, MACB 92659; Lubia, Altos de Lubia, 07-IX-2003, *A. R. Burgaz*, MACB 90569; Matalebreras, 10-IX-1991, *T. Ahti & A. R. Burgaz*, MACB 45687; Matalebreras, 13-IX-2003, *A. R. Burgaz*, MACB 90623; Olvega, 05-IX-1984, *A. R. Burgaz*, MACB 45690; Pto. de Santa Inés, vertiente norte, 07-IX-2003, *A. R. Burgaz*, MACB 91700; Vozmediano, 04-IX-1984, *A. R. Burgaz*, MACB 41485; Tarragona: Cardó, Sª de Cardó, 04-IV-2003, *A. R. Burgaz*, MACB 90571; Cardó, Sª de Cardó, 09-IV-2003, *A. R. Burgaz*, MACB 90630; Coll de Gua, 25-VI-1992, *A. R. Burgaz*, MACB 90626; Conca de Barberá, Poblet, 21-V-1998, *A. R. Burgaz*, MACB 91697; Gavadá, Sª de la Talaida, 05-X-2004, *A. R. Burgaz*, MACB 90568; Les Garrigues, La Pobla de Cérvoles, 22-V-1998, *A. R. Burgaz*, MACB 90574; Les Garrigues, La Pobla de Cérvoles, 22-V-1998, *A. R. Burgaz*, MACB 90628; Rasquera, Sª de Cardó, 09-IV-2003, *A. R. Burgaz*, MACB 90627; Sª de Montenegreto, 25-VI-1992, *A. Herrero*, MACB 90508; Valle de Castelfullit (Bimbodi), cerca de Poblet, 11-IX-1988, *M. Casares*, GDA; Vilanova de Prades. Sª de Prades, 28-VII-2003, *A. R. Burgaz*, MACB 90572; Teruel: Albarracín, 12-VI-1991, *A. R. Burgaz & E. Fuertes*, MACB 41480; Albarracín, Sª de Albarracín, bco. del Navazo, 18-V-1996, *G. Aragon, A. Herrero & I. Martínez*, MACB 90512; Cucalón, Sª de Fonfría, 23-VI-1992, *A. R. Burgaz*, MACB 90605; Ejulve, Llano de Villaseco, 24-VI-1992, *A. R. Burgaz*, MACB 90523; El Parrisal, Beceite, P. Nat. de los Puertos de Beceite, rio Matarraña, 08-IV-2003, *A. R. Burgaz*, MACB 90575; Frías de Albarracín, 12-VII-1991, *A. R. Burgaz*, MACB 45685; Monterde, 13-VI-1991, *A. R. Burgaz & E. Fuertes*, MACB 41495; Noguera, Sª de Albarracín bco. de los Polos o de las Fuentes, 18-V-1996, *G. Aragon, A. Herrero & I. Martínez*, MACB 90525; Puerto de Bronchales, 12-VI-1991, *A. R. Burgaz*, MACB 45688; Puerto de San Just, 23-VI-1992, *A. R. Burgaz*, MACB 90521; Puerto de Valdecuenca, Sª de Albarracín, 12-VI-1991, *A. R. Burgaz & E. Fuertes*, MACB 41492; Puerto de Valdelinares, 13-VI-1991, *A. R. Burgaz*, MACB 41494; Puerto de Valdelinares, Sª de Gudar, 13-VI-1991, *A. R. Burgaz & E. Fuertes*, MACB 41484; Puerto de Valdemeca, 20-X-1997, *A. R. Burgaz*, MACB 91681; Puerto Majalinos, 23-VI-1992, *A. R. Burgaz*, MACB 90524; Sª Cucalón, Fonfria, 23-VI-1992, *A. R. Burgaz*, MACB 90526; Subida al Puerto de Javalambre, 14-V-1991, *A. R. Burgaz & E. Fuertes*, MACB 41493; Tronchón, 24-VI-1992, *A. R. Burgaz*, MACB 90522; Toledo: Belvis de la Jara, 26-XII-2004, *R. Pino-Bodas*, MACB 91698; Consuegra, 05-XI-2004, *A. R. Burgaz*, MACB 90575; El Mazo, 15-II-2006, *A. R. Burgaz*, MACB 92663; La Iglesuela, 23-III-1996, *A. R. Burgaz*, MACB 90576; Los Navalucillos, Valle del Chorro, 11-X-1992, *Martinez*, MACB 90577; Marrupe, *R. Pino-Bodas*, MACB 90580, MACB 90580; San Pablo de los Montes, Montes de Toledo, 22-V-2002, *A. R. Burgaz & Mª A. Carrasco*, MACB 90590; San Roman de los Montes, 01-I-2005, *R. Pino-Bodas*, MACB 90581; Urda, Sª Morrones, subida antena TV, 05-XI-2004, *A. R. Burgaz*, MACB 90587; Valencia: Buñol, Sª de Malacas, Siete Aguas, 17-II-1996, *A. R. Burgaz*, MACB 90591; Cofrentes, 28-V-1996, *A. R. Burgaz & I. Martínez*, MACB 90629; Valladolid: Castromonte, embalse de la Santa Espina, 01-IX-2000, *A. R. Burgaz*, MACB 90631; Pedrajas de San Esteban, 20-VII-2006, *A. R. Burgaz*, MACB 102926; Zamora: Almaraz de Duero-Zamora, Km 10, 07-IX-1998, *A. R. Burgaz, S. Casas & I. Rodriguez Lopez,* MACB 70175; La Tabla, 07-X-1997, *G. Aragón, A. R. Burgaz & A. Terrón*, MACB 70174; Las Enillas, 07-IX-1998, *A. R. Burgaz, Casas S. & I. Rodriguez Lopez,* MACB 70177; Pereruela, arroyo del Zape, 07-IX-1998, *A. R. Burgaz, S. Casas & I. Rodriguez Lopez,* MACB 70176; Villafáfila, 26-XII-1992, *A. Herrero*, MACB 90423; Villalazán, 06-X-1997, *A. R. Burgaz*, MACB 70235; Zaragoza: Alfarajín, 10-X-90, *M. Casares*, GDA 2077; Circo de San Miguel, Sª del Moncayo, 13-IX-2003, *A. R. Burgaz*, MACB 90595; Embid de la Ribera, 04-VI-2003, *A. R. Burgaz*, MACB 90592; Sestrica, 20-IX-2003, *A. R. Burgaz*, MACB 90597; Baleares Islands: Mallorca, Algaida, camí vell d' Algaida, 10-I-2006, *A. R. Burgaz*, MACB 92682; Andratx, coll de Sa Gram Mola, Sª de Tramuntana, 12-I-06, *A. R. Burgaz*, MACB 92725; Escorca, Menut II, Sª de Tramuntana, 11-I-2006, *A. R. Burgaz*, MACB 92719; Felanitx, Santuario de San Salvador, 12-I-2006, *A. R. Burgaz*, MACB 92715; Fornalutx, Sa Comuna, Sª de Tramuntana, 9-I-2006, *A. R. Burgaz*, MACB 92683; Inca, 11-I-2006, *A. R. Burgaz*, MACB 92680; Llucmajor, Cala Blava, 12-I-2006, *A. R. Burgaz*, MACB 92716; Llucmajor, Cap Blanc, 12-I-2006, *A. R. Burgaz*, MACB 92717; Pollença, carretera de cabo Formentor, 11-I-2006, *A. R. Burgaz*, MACB 92727; Pollença, El Mal Pas, 11-I-2006, *A. R. Burgaz*, MACB 92720; Sa Pobla, 11-I-2006, *A. R. Burgaz*, MACB 92726; Santa Margarita, 10-I-2006, *A. R. Burgaz*, MACB 92681; Santa Margarita, San Serra de Marina, 10-I-2006, *A. R. Burgaz*, MACB 92679; Ses Salines, cabo de Ses Salines, 13-I-06, *A. R. Burgaz*, MACB 92714; Valldemossa, El Encinar, Sª de Tramuntana, 11-I-2006, *A. R. Burgaz*, MACB 92720; **Sweden**: Bohuslän: Tjärnö, islet Inre Tenskär, 10-VIII-1985, *T. Ahti 6 S. Hyvönen*, H; Öland: Böda parish, 1.5 Km N.N.E. of Getterum, 9-VII-1962, *R. Santesson*, H; Böda sn., Mensalvaret, 3-VIII-1983, *R. Skytén*, H; Böda, Mensalvret, 3-VIII-1983, *O. Vitikainen*, H; Sverige: Skane, Ahs-Yngsjö, Gropahalet, 26-VII-1979, *C. A. haeggström*, H; Ol. Resmo alloau, 16-VI-1910, *D. Rietz*, H; **United Kingdom**: Scotland: East

Lothian, Nort Berwich, IX-2006, *B. Coppins*, MACB 95602; Haughnond Hill, Shropshire, *W. A. Leighton*, H; Suffolk: Lakenheath Warren, 30-I-1965, *D. Hawsworth*, H.

***Cladonia furcata* (Huds.) Schrad.**

Andorra: Vall d'Incles, río Jucar, refugio de Sisqueró, 3-VII-1996, *A. R. Burgaz & I. Martínez*, MACB 98074; **Australia**: Australian Capital Territory: Aranda Bushland, Canberra, 7-XI-1998, CANB 740213; New South Wales: South Coast, west-facing road cut, Merimbula Tathra road, 5 Km N of Merimbula, V-1998, *S. Hammer*, CANB 619976; Tidbinbilla Nature Reserve, Fishing Gap Trail, between loop road and firs culvert/bridge, 21-III-1999, *P.C. Heyligers*, CANB 740296; West-facing road cut, Merimbula Tathra Road, 5 Km N of Merimbula, V-1998, *S. Hammer*, FH; Queensland: Binna Burra, Lamigton National Park, VII-2000, *S. Hammer*, FH; Moreton, Binna Burra, Lamnington National Park, VII-2000, *S. Hammer*, CANB 654481; Moreton, Binna Burra, Lamnington National Park, VII-2000, *S. Hammer*, CANB 654480; On rotting wood, Binna Burra, Limington National Park, VII-2000, *S. Hammer*, FH; Vicinity of O'Reilly's Guesthouse, Lamington National Park, VII-2000, *S. Hammer*, FH; Victoria: Brock Monument immediately E from the junction of Monument Road with Woodend-Wallen Road, c. 6Km W from Romsey township and c. 8 KmE from Hanging Rock, 12-IV-2000, *V. Stajsic*, CANB 56115; Errindura National Park, Errindura Saddle, sunny clearing near Boardwalk, V-1998, *S. Hammer*, FH; Gippsland region, Errindura National Park, Errindura Saddle, 20 Km SSE of Bonang, V-1998, *S. Hammer*, CANB 619977; Princes Highway, 1 Km of Genoa, V-1998, *S. Hammer*, FH; **Austria**: Steiermark: Alpes orientales, Nördliche Kalkalpen, Sª de Hochschwab, valle de Brunntal SE del pueblo Wildalpen, 20-IX-1996, *A. R. Burgaz, J. Hafellner & I. Martínez*, MACB 65999; Seetaler Alpen, 10 Km WNW of Obdach, road from Schmelz to Winterleitenhütte, 30-VII-2005, *W. Obermayer*, UPS L-159998; Tirol: Stubaier Alpen: Steinach am Brenner, nördl. von der Velperquelle, 24-VIII-1964, *M. Steiner*, MA-lichen 504; **Brasil**: Estado de Paraiba: Mamamguape, V-1992, *E. C. Pereira*, MACB 46632; **Canada**: Ontario: Lake Opeongo, Algonquin Park, Ont, 19-IX-1951, *R. F. Cain*, L 794560; **Croatia**: Dubrovnik-Neretva: Zamaslina, península de Pelsejac, calizas, *A. R. Burgaz*, MACB 101098; Primorje-Gorski Kotar: Istria, Rijeka, Joegoslavië, omgeving Ryeka, 4-VI-1975, *J. H. Lestwaart*, L 669345; **Denmark**: Capital Region of Denmark: Bornholm, Blykobbe Plantage, Skovly, 13-VII-2001, *E. S. Hansen & J. Hansen*, H; Bornholm, Gudhjem near "Serpentinervejen", 17-IX-2005, *E. S. Hansen*, H; Bornholm, Hasle Lystoskov, 9-VII-2001, *E. S. Hansen*, H; Bornholm, Knudsnaes S of Allinge, 21-VII-1999, *E. S. Hansen*, H; Bornholm, Listed, 12-VII-2002, *E. S. Hansen*, H; Bornholm, Sandkas, 19-VI-1996, *E. S. Hansen*, H; Bornholm, Svaneke, 9-VII-2001, *E. S. Hansen*, H; Faroe Island: Streymoy, Törshvn, N part of the city, N of the harbour, 14-V-2007, *L. H. Väre*, H; Jylland: Holmsland Klit, Bjerregard, 27-VII-2005, *E. S. Hansen*, H; Kandestederne, 26-V-2000, *E. S. Hansen*, H; Kandesterne, 26-VI-2000, *E. S. Hansen*, H; Mols, Strandkaer, 5-X-2002, *E. S. Hansen*, H; Southeast of Hulsig, 17-IV-1999, *S. N. Christensen*, H; Syddanmark: Fano, Nordby, 3-VII-2004, *E. S. Hansen*, H; Zealand: Asserbo Plantage, 24-X-2005, *P. Corfixen, S. Christensen & E. S. Hansen*, H; Eskebjerg, Vesteriyng, 8-IV-2002, *R. S. Larsen, P. Corfixen, S. N. Christensen & E. S. Hansen*, H; Melby Overdrev, 24-X-2005, *P. Corfixen, S. Christensen & E. S. Hansen*, H; Mon, Ulvshale, 8-VI-1998, *A. V. Larsen, P. Corfixen, S. N. Christensen & E. S. Hansen*, H; Saltbaek Vig, Raklev, 10-VI-1970, *M. S. Christiansen*, H; Tisvilde Hegn, Lerbjerg, 8-XII-2004, *P. Corfixen, S. Christensen & E. S. Hansen*, H; Venslev, W of Naestved, 11-VIII-1969, *M. S. Christiansen*, H; **Finland**: Åland Islands: Alandia, Brändö, Lilla Flöjskär, 23-IV-2004, *M. Sternberg*, H; Alandia, Brändö, Porsskär, Ytterskäar, 24-V-2003, *M. Sternberg*, H; Alandia, Kumlinge SE skärgard, Salungarna, 20-VII-2003, *M. Sternberg*, H; Alandia, Kumlinge, Enklinge, Blacksund, 1-IX-2002, *M. Sternberg*, H; Alandia, Kumlinge, Ulvingen, 20-VII-2003, *M. Sternberg*, H; Central Finland: Joutsa, along Kangasniemen tie in the E side of Valklampi near electrical installations, 28-VI-2007, *P. Alanko*, H; Central Finland: Punkaharju, Vuoriniemi, Putikko, Kallioleikauksen, 3-VIII-2003, *T. Rintanen*, H; Finland Proper: Ab, Pargas, Stortervolandet, Hällmarkstallskog, 14-VII-1979, *R. Skytén*, H; Korpo, Avensor, Kälklot, 5-VII-1988, *R. Skytén*, H; Päijänne Tavastia: Asikkala, Kalkkisten kanava, 21-III-2007, *V. Haikonen*, H; Asikkala, Maakeskenlahti, Huuhinsaari, 1-IV-2009, *V. Haikonen*, H; Asikkala, Pirunmäki, 17-X-2002, *V. Haikonen*, H; Asikkala, Viitaila, Kiikkilä, Päijänmetunnelin, 12-XI-2007, *V. Haikonen*, H; Hämeenko, Ilolanharjut, 21-VIII-2008, *V. Haikonen*, H; Hämeenkoski, Laguksen muistomerkki, 21-VIII-2008, *V. Haikonen*, H; Hämeenkoski, Laguksen muistomerkki, 21-VIII-2008, *V. Haikonen*, H; Heinola, Hujansalo, Juhola, 16-XI-2008, *V. Haikonen*, H; Hollola, Kalliola, Sudenpesänmäki, 9-VIII-2008, *V. Haikonen*, H; Hollola, Paimela, Paimelanvuori, 17-X-2008, *V. Haikonen*, H; Jämsänkoski, Juokslahti, 26-IX-2003, *V. Haikonen*, H; Kalvola, Kothajärvi, Pastinkangas, 19-IV-2008, *H. Väre*, H; Lammi, Porraskoski, Haukkavuori, 18-IV-2002, *V. Haikonen*, H; Litti, Vuolenkoski, Sahanniemi, 25-X-008, *V. Haikonen*, H; Nastola, Seesta, 13-X-2002, *V. Haikonen*, H; Nastola, Villähde, Tapiola, 8-XI-2002, *V. Haikonen*, H; Padasjoki, Kasiniemi, Majaniemi, 5-IV-2002, *V. Haikonen*, H; Padasjoki, Maakeski, Möysälänkalliot, 5-V-2009, *V. Haikonen*, H; Sysmä, Karilanmaa, Kaapparinharju, 24-IV-2008, *V. Haikonen*, H; Sysmä, Vehkasalo, Huhkaimenvuori, 5-X-2008, *V. Haikonen*, H; Pirkanmaa: Tampere, Teisko, Kulhanvouri, 24-X-1998, *M. Käantönen*, H; Uusimaa: Nurmijärvi, Perttula, Äijänkallio, 29-IX-1995, *T. Ahti, A. R. Burgaz, I. Martínez & O. Vitikainen*, MACB 96303; Porvoo rural parish, island Emäsalo, Varlaxudden, 10-X-1995, *T. Ahti, A. R. Burgaz, I. Martínez & O. Vitikainen*, MACB 96304; **France**: Alsace: Haut-Rhin, Orbey, Vogezen, omgeving Orbey, 7-VIII-1975, *J. H. Lestwaart*, L 669267; Pyrenées atlantiques: 8 Km despues de Banca, foret d'Hayra, 27-X-1993, *J. Etayo*, MA-lichen 4456; **Indonesia**: Java, *J. E. Fepsmann*, H; **Iran**: Golestam: Gorgan, Galuogah toward Hezajerib area, 2-IV-2004, *M. Ebrahimi*, H; Gorgan, Golestan National Park, Tangheh gol toward to Golzar around road, 30-V-1999, *M. Sohrabi*, H; **Ireland**: Munster: Co. Clare, near Fanmore S. of Black Head, 19-VI-1971, *Rheno-Trai*, L 794566; **Netherlands**: Utrecht: Leusderheide, Leusden, III-2009, *L. Spier*, Spier 17769; Leusderheide, Leusden, III-2009, *L. Spier*, Spier 17768; **New Caledonia**: South Province: Roadside South of Noumea, XII-1998, *S. Hammer*, FH; **New Zealand**: North Island, Northland: Bear Omahuta Forest Sanctuary, vicinity of Umawera, I-2000, *S. Hammer*, CANB 654507; Near Omahuta forest Sanctuary, vicinity of Umawera, I-2000, *S. Hammer*, FH; South Island, Canterbury: Vicinity of Lewis Pass near Maruia Hot Springs, XII-2000, *S. Hammer*, CANB 653788; Vicinity of Lewis pass near Maruia Hot Springs, XII-2000, *S. Hammer*, FH; Vicinity of Lewis pass near Maruia Hot Springs, XII-2000, *S. Hammer*, FH; Whisky Creek Trail, Mt. Arthur National Park, I-1999, *S. Hammer*, FH; South Island, Nelson: Sharland Creek Entrance Path, vicinty of Nelson, XII-1998, *S. Hammer*, CANB 565833; Sharland Creek Entrance Path, vicinity of Nelson, XII-1998, *S. Hammer*, FH; Sharland's Creek Entrance Road, vicinity of Nelson, XII-1998, *S. Hammer*, FH; Southland, Hmer Saddle pulloff, Highway 94 just E of Homer Tunnel, I-2000, *S. Hammer*, CANB 667724; Southland, Manuka heath, Monowai Road, vicinity of Borland Bog, I-2000, *S. Hammer*, FH; Vicinity of Heaphy Track ca. 15 Km N. of Karamea, I-2000, *S. Hammer*, FH; Vicitiny of Heaphy Track ca 15 Km N of Karamea, XII-2000, *S. Hammer*, CANB 653804; South Island, West Coast: Haast-Jackson Bay Road just east of Waiatoto Lagoon, ca 5 Km S. of Hannahs Clearing, I-1999, *S. Hammer*, CANB 565832, FH; Homer Saddle pulloff, Hwy. 94 just E. of Homer Tunner, I-2000, *S. Hammer*, FH; **New Zealand**: South Island, West Coast: Vicinity of Heaphy Track, N of Karamea, XII-2000, *S. Hammer*, FH; Westland, Highway 6 southbound, vicinity of Nelson, XII-1998, *S. Hammer*, CANB 653813; Westland, Hwy. 6 sothbound, vicinity of Nelson, XII-1998, *S. Hammer*, FH; Whiskey Creek Trail, Mt. Arthur, I-1999, *S. Hammer*, CANB 56831; Whiskey Creek Trail, Mt. Arthur, I-1999, *S. Hammer*, FH, CANB 653741; **Philippines**: Luzon: Mt Pulog, 27-I-1968, *M. Jacobs*, L 794563; **Portugal**: Algarve:

Alferce, Sª de Monchique, 8-XII-2006, *A. R. Burgaz*, MACB 94355; Aljezur, Praia do Monte Clérigo, 9-XII-2006, *A. R. Burgaz*, MACB 94354; María Vinagre, hacia la Bahía dos Tiros, 9-XII-2006, *A. R. Burgaz*, MACB 95410; María Vinagre, hacia la Bahía dos Tiros, 9-XII-2006, *A. R. Burgaz*, MACB 94350, MACB 94353; Monchique, Sª de Monchique, subida a Foia, 8-XII-2006, *A. R. Burgaz*, MACB 94356; Alto Alentejo: Montinho, P. Nat. São Mamede, 11-I-2004, *A. R. Burgaz*, MACB 91083; P. Nat. São Mamede, alto del pico São Mamede, 10-I-2004, *A. R. Burgaz*, MACB 91088, MACB 91087; P. Nat. São Mamede, subida al pico São Mamede, 10-I-2004, *A. R. Burgaz*, MACB 91084; Pégões, Monte das Piçarras, 9-I-2004, *A. R. Burgaz*, MACB 91028; Baixo Alentejo: Cavalheiro, cabo Sardão, 9-XII-2006, *A. R. Burgaz*, MACB 94352; Beira Alta: Busaco, 22-II-1990, *A. R. Burgaz*, MACB 66896; Castelo Mendo, 1-V-2007, *A. R. Burgaz*, MACB 95409; Covilhã, Sª da Estrela, P. Nat. de Sª da Estrela, 2-V-2007, *A. R. Burgaz*, MACB 95486; Estrela, Estrela, Ribeira da Pragueira, 22-X-1995, *N. Marcos, P. Navarro & E. Munín*, MACB 66889; La Torre, Sª da Estrela, P. Nat. de Sª da Estrela, 3-V-2007, *A. R. Burgaz*, MACB 95050; Manteigas, Sª da Estrela, P. Nat. de Sª da Estrela, cabeza del río Zêzere, 2-V-2007, *A. R. Burgaz*, MACB 95900, MACB 95056; Manteigas, Sª da Estrela, P. Nat. de Sª da Estrela, poço do Inferno, 2-V-2007, *A. R. Burgaz*, MACB 95049; Penhas da Saúde, Sª da Estrela, P. Nat. de Sª da Estrela, 2-V-2007, *A. R. Burgaz*, MACB 95051, MACB 95487; Penhas Douradas, Sª da Estrela, P. Nat. de Sª da Estrela, 2-V-2007, *A. R. Burgaz*, MACB 95819, MACB 95052; Penhas Douradas, Sª da Estrela, P. Nat. de Sª da Estrela, 2-V-2007, *A. R. Burgaz*, MACB 95057; Rabacal, Sª de Sicó, 28-I-1996, *A. R. Burgaz & I. Martínez*, MACB 66898; Sabugueiro, Lagoa Comprida, Sª da Estrela, P. Nat. de Sª da Estrela, 3-V-2007, *A. R. Burgaz*, MACB 94897; Viseu, San Miguel, 26-I-1996, *A. R. Burgaz & I. Martínez*, MACB 66891; Beira Litoral: Aveiro, Costa Nova, 3-IV-1998, *T. Ahti & A. R. Burgaz*, MACB 66902; Coimbra, Coja, Serra do Açor Fraga da Pena, 8-III-1996, *N. Marcos*, MACB 91027; Figueira da Foz, Mata Nacional das Dunas de Quiaios, 14-VI-2007, *S. A. Rodrigues*, MACB 95488; Luso, Bussaco a 2 Km de Cruz Alta, 3-IV-1998, *T. Ahti & A. R. Burgaz*, MACB 66894; Luso, Bussaco, Cruz Alta, 3-IV-1998, *T. Ahti & A. R. Burgaz*, MACB 66895; Mealhada, Luso, Bucaco Cruz Alta, 28-I-1996, *A. R. Burgaz & I. Martínez*, MACB 66893; Sª do Arçor, a 1 Km de Benfeite, 29-III-1998, *T. Ahti & A. R. Burgaz*, MACB 66901; Sª do Arçor, Fraga de Pena, Barroco de Degrainhos, 1.5 Km SSE de Benfeite, 29-III-1998, *T. Ahti & A. R. Burgaz*, MACB 66899; Sª do Arçor, mata de Margaraça 2.5 Km de Benfeite, 29-III-1998, *T. Ahti & A. R. Burgaz*, MACB 66900; Sª de Sintra, Monasterio de Peninha, 10-I-2004, *A. R. Burgaz*, MACB 91072; Estremadura: Sintra, Serra de Sintra subiendo a la Cruz Alta, en el Castelo dos Mouros, 16-III-1997, *A. R. Burgaz*, MACB 66890; Minho: Sapiaos, 12-VIII-1994, *A. R. Burgaz*, MACB 66897; Serra Amarela, Ermida, 16-VI-1995, *A. R. Burgaz, I. Martínez & P. Navarro*, MACB 91108; Serra do Soãjo, Soãjo-Adrão, 17-VI-1995, *A. R. Burgaz, I. Martínez & P. Navarro*, MACB 66892; Ribatejo: Santarém, P. Nat. Serra de Aires y Candeeiros, 15-II-2001, *P. Pinho*, MACB 91026; Trás Os Montes: Alimonde, Sª da Nogueira, valle de Alimonde, 6-IX-2006, *A. R. Burgaz*, MACB 94131; Alrrededores Señora da Serra, Sª de Nogueira, 20-II-2005, *A. R. Burgaz*, MACB 92890, MACB 91095; Bragança, Sª de Nogueira, 4-X-1998, *N. Marcos & I. Cubero*, MACB 91004, MACB 91005; Izeda, valle del río Sabor, 5-IX-2006, *A. R. Burgaz*, MACB 93863; Izeda, valle del río Sabor, 5-IX-2006, *R. Pino-Bodas*, MACB 94031, MACB 94026; Lagoa, valle del río Sabor, 5-IX-2006, *R. Pino-Bodas*, MACB 93688; Montezinho, Sª de Montezinho, 8-IX-2006, *R. Pino-Bodas*, MACB 93699; Montezinho, Sª de Montezinho, 8-IX-2006, *A. R. Burgaz*, MACB 94129; Montezinho, Sª de Montezinho, 8-IX-2006, *R. Arroyo & E. Seriñá*, MACB 93451; Nogueira, Sª de Nogueira, 20-II-2005, *A. R. Burgaz*, MACB 102839; Rebordãos, Sª da Nogueira, 6-IX-2006, *A. R. Burgaz*, MACB 93864; Rebordaos, Sª da Nogueira, 6-IX-2006, *R. Pino-Bodas*, MACB 94032; Rebordãos, Sª da Nogueira, 6-IX-2006, *R. Pino-Bodas*, MACB 94030, MACB 95408; **Russia**: Karelia Republic: Karelia ladogensis, Sortavala, 7 Km S of the harbour, Riekkalansaari, Kekrinlahti, 14-VI-1997, *O. Vitikainen*, H; Karelia olonetsensis, Kalajoki, Rybreka, 8-VIII-1898, *A. K. Cajander*, H; Karelia olonetsensis, Lososinnoje, Järven ranta, guangfly, 21-VI-1898, *A. K. Cajander*, H; Republic of Karelia: Kolplace, Vepskaya volost', Kvarzitny, 26-VII-2004, *M. A. Oameeba*, H; Krasnoyard: N of Central Siberia, Severnaya Zemlya Archipelago, N part of Bol'shevik Is., W coast of Akhmatov Bay, 7 Km SW of Bazavaya River mouth, 15-VII-1996, *M. Zhurbenko*, H; Krasnoyarsk Krai: N of Central Siberia, Severmaya Zemlya Archipelago, N part of Bol'shevik Is., W coast of Akhmatov Bay, 7 Km SW of the Bazavaya River mouth, 18-VII-1996, *M. Zhurbenko*, H; Leningrad: Karelia australis, Vyborg distric, Berëzovye Island Reserve, Bol'shoy Berëzovyy Island, Krasnyy Ostrov, S side of Kuznetskaya Bay, 12-VIII-2008, *T. Ahti*, H; Sakha Republic: Kangalassy, National Park Lenskie Stolby, south bank of Lena River, 0.5-1 km S of mouth of Labyiya River, 30-VI-2002, *T. Ahti*, H; Kangalassy, National Park Lenskie Stolby, south bank of Lena River, 0.5-1 km S of mouth of Labyiya River, 30-VI-2002, *T. Ahti*, H; Namski District, Khomustakh, ca. 5 km S, on high bank of Lena River valley, 11-VIII-2005, *T. Ahti & A. P. Efimova*, H; Sakhalin Oblast: Far east, Kurile Island, Kunahir Island, Golovin, 19-VIII-2003, *S. Abrahamczyk*, H; Madeira Island: Corticeiras, Baco da Corrida-Encumeada, along mountain path near Basso de Ares, 11-II-2004, *P. Alanko*, H; Montado de Pessegueiro, Paul da Serra, I-2003, *M. P. Jones*, H; Paul da Serra W, along Ribeiro Janela, NW of Rabaçal, 7-X-2005, *H. Väre*, H; **Spain**: Asturias: Beleño, 9-IV-1991, *A. R. Burgaz & N. Marcos*, MACB 44248; Felechosa, Foces del Pino, 5-VIII-2008, *A. R. Burgaz*, MACB 91076; Genestoso, 15-VIII-1994, *T. Almaraz, G. Aragón, J. Castillo & I. Martínez*, MACB 92600; La Raya, Reserva Nacional de Mampodre, Puerto de San Isidro, 22-VII-2006, *A. R. Burgaz*, MACB 94818, MACB 93513; Llamardal, subida Puerto de Somiedo, 8-VIII-2003, *A. R. Burgaz*, MACB 91077, MACB 91043; Llamardal, subuda al Puerto de Somiedo, 8-VIII-2003, *A. R. Burgaz*, MACB 91053; Puerto Ventana, 25-X-1994, *A. R. Burgaz, N. Marcos & P. Navarro*, MACB 91048; Somiedo, 25-X-1994, *A. R. Burgaz*, MACB 91016; Vega de Rengos, Puerto de Rañadairo, 24-X-1994, *A. R. Burgaz & I. Martínez*, MACB 97263; Ávila: Alto de los Leones, collado de la Mina, 8-V-2005, *A. R. Burgaz*, MACB 94898, MACB 95053; Candelario, P. Nat. de Candelario, subida a El Travieso, 1-VII-2007, *A. R. Burgaz*, MACB 95330; Hoyos del Espino, Sª de Gredos, subida al Puerto de Canencia, vertiente N, 29-VII-1997, *A. R. Burgaz, N. Marcos & P. Navarro*, MACB 91092; Monsalupe, 1-V-2007, *A. R. Burgaz*, MACB 95406; Navamojada, 10-VI-1992, *A. R. Burgaz*, MACB 102803; Ojos-Albos, 5-XI-2007, *A. R. Burgaz*, MACB 92841; Ojos-Albos, 5-XI-2007, *A. R. Burgaz*, MACB 92842; Ojos-Albos, afloramientos cuarcíticos, *A. R. Burgaz*, MACB 102805; Peguerinos, alrrededores del campamento "Peñas Blancas", 10-V-1998, *A. R. Burgaz*, MACB 91104; Piedrahita, 21-XI-2004, *A. R. Burgaz*, MACB 102820; Pto. de Villatoro, 21-XI-2004, *A. R. Burgaz*, MACB 102949, MACB 91071, MACB 91118; San Bartolomé de Bejar, 1-VII-2007, *A. R. Burgaz*, MACB 95407; Barcelona: El Bages, Castellfollit del Boix, Sª de Rubio, 20-V-1998, *A. R. Burgaz*, MACB 91103; El Brull, P. Nat. del Montseny, coll de Formic, 16-VIII-2006, *A. R. Burgaz*, MACB 94438; Barcelona: Monseny, P. Nat. del Montseny, Turó de l'Home, 16-VIII-2006, *A. R. Burgaz*, MACB 94819; Burgos: Basconcillos del Tozo, 9-VI-1988, *A. R. Burgaz & E. Fuertes*, MACB 44247; Berberana, Puerto de Orduña, monte de Santiago, 24-VIII-1994, *A. Herrero*, MACB 91018; Covarrubias, Sª de Covarrubias, 6-IX-2003, *A. R. Burgaz*, MACB 91032; Nelia, Sª de Nelia, 6-IX-2003, *A. R. Burgaz*, MACB 91066, MACB 91050; Nelia, Sª de Nelia, Laguna Larga, 6-IX-2003, *A. R. Burgaz*, MACB 91075; Quintanar de la Sª, 6-IX-2003, *A. R. Burgaz*, MACB 91073, MACB 91049; Santa Cruz del Valle Urbión, Sª de la Demanda, 2-IX-1991, *I. Martínez, A. Herrero*, MACB 44241; Urrez, Sª de Mencilla, 12-III-2008, *A. R. Burgaz*, MACB 97287; Villasur de Herreros, Sª de San Millán, valle río Arlanzón, 13-III-2008, *A. R. Burgaz*, MACB 97288; Cáceres: Acebo, Sª de Gata, pico Jálama, 2-IV-1996, *G. Aragón, A. Herrero & I. Martínez*, MACB 91121; Guadalupe, Sª de Guadalupe, subida al pico Villuercas, 4-V-2007, *A. R. Burgaz*, MACB 95054; Hoyos, Sª de Santa Olalla, 6-IV-1996, *G. Aragón, A. Herrero & I.Martínez*, MACB 91035; Las Mestas, Sª de las Mestas, valle del río Batuecas, 6-XI-2005, *A. R. Burgaz*, MACB 92962; NavatraSª, Sª de la Palomera, 5-V-2007, *A. R. Burgaz*,

MACB 95055; San Martín de Trevejo, Sª de Gata, 26-VII-1997, *A. R. Burgaz*, MACB 91093; San Martín de Trevejo, Sª de Gata, rio de la Vega, 4-IV-1996, *G. Aragón, A. Herrero & I.Martínez*, MACB 91111, MACB 91037, MACB 92844; Villareal de San Carlos, Parque de Montfragüe, Sª de las Corchuelas, 5-IV-1991, *F.Sarrión*, MACB 45737; Cádiz: Alcalá de los Gazules, El Picacho, P. Nat. de los Alcornocales, 21-IX-2004, *A. R. Burgaz*, MACB 91115, MACB 91094; Algeciras, P. Nat. de los Alcornocales, Alto de la Miel, 27-XII-1992, *I. Martínez*, MACB 96210; Barbate, El Soto, 25-V-1997, *A. R. Burgaz & N. Marcos*, MACB 91102, MACB 92843; Los Barrios, a Tiradero, 30-XII-1992, *I. Martínez*, MACB 91040; Sª del Aljibe, 4-IV-1991, *F. Sarrión & I. Martínez*, MACB 44343; Sª del Niño, 1-III-1997, *F. Saiz*, MACB 91110; Ubrique, P. Nat. de los Alcornocales, 18-XI-2005, *S. Silvestre*, MACB 96004; Vejer de la Frontera, 12-X-1995, *A. R. Burgaz*, MACB 64877; Cantabria: Camaleño, Invernales de Mato, 8-VI-1994, *A. R. Burgaz*, MACB 96658; Campoo de Cabuerniga, monte Canal del Infierno, 29-VI-1996, *I. Martínez*, MACB 91009; Cosgaya, Sª Mediana, subida a Coriscao, 14-X-2005, *G. Aragón, A. García & M. Prieto*, MACB 95894; Camaleño, 8-VI-1994, *A. R. Burgaz*, MACB 91044; Camaleño, P. N. P. E., Pido, 15-XI-2003, *G. Amo, A. R. Burgaz, I. Martínez & M. Otalora*, MACB 89749; Campoo de Cabuerniga, río Saja Espinilla-Saja, 1-IV-1994, *G. Aragón & I. Martínez*, MACB 91036; Comunidad de Cabuérniga, valle del río Saja, 1-IV-1994, *G. Aragón & I. Martínez*, MACB 91045; Ciudad Real: Fuencaliente, Arroyo Robledillo de las Hoyas, 5-II-1997, *A. R. Burgaz, I. Martínez & F. Sarrión*, MACB 91041, MACB 91046; Fuencaliente, cerca del río Cereceda, 4-I-1990, *F. Sarrión*, MACB 37271; Villarubia de los Ojos, Sª de la Cueva, Puerto de los Santos, 5-XI-2004, *A. R. Burgaz*, MACB 91120; Cuenca: San Clemente, 11-V-2004, *A. R. Burgaz*, MACB 102797, MACB 102789; Sª de Valdemeca, arroyo Verrtiente, 29-V-1996, *A. R. Burgaz & I. Martínez*, MACB 91017; Gerona: Alp, Supermolina, Sª del Cadí, 2-VII-1996, *A. R. Burgaz & I. Martínez*, MACB 91068; La Escala, 20-VII-1960, *J. Hernig*, L 794558; Queralbs, Vall de Núria, 18-VIII-2006, *A. R. Burgaz*, MACB 95818; Sant Martí Vell, El Girones, Santuario els Angels, 5-IX-2007, *A. R. Burgaz*, MACB 95927; Santa Pau, volcán de Santa Margarita, 2-I-1992, *A. Herrero*, MACB 44233; Setcases, estación de esqui Vallter 2000, 17-VIII-2006, *A. R. Burgaz*, MACB 94539, MACB 96659; Torroella de Montgrí, El Baix Empordan, Les Dunes, 6-IX-2007, *A. R. Burgaz*, MACB 95926; Vallfogona del Ripollés, 10-XII-1995, *G. Aragón, A. Herrero & I. Martínez*, MACB 91112, MACB 92845; Granada: Loja, Sª de Loja, 17-11-2005, *A. R. Burgaz*, MACB 102834, MACB 102815; Guadalajara: Bustares, Sª de Alto Rey, 23-VIII-2003, *A. R. Burgaz*, MACB 91065; Cantalojas, barranco río Lillas, P. Nat. Tejera Negra, 23-VIII-2003, *A. R. Burgaz*, MACB 91067, MACB 91056; Gascueña de Bornoba, Sª de Alto Rey, 23-VIII-2003, *A. R. Burgaz*, MACB 91060; Sigüenza, 12-X-2002, *A. R. Burgaz*, MACB 102790; Valle de lso Canutos, Peñalba de la Sª, 6-VIII-2002, *G. Amo de Paz*, MACB 91022; Huesca: Argüis, Parque de Guara, Sª de la Gabardiella, antenas de TVE, 22-VIII-2006, *A. R. Burgaz*, MACB 94139; Astún, valle de Canfranc, 28-VII-2000, *A. R. Burgaz*, MACB 91080; Astún, valle de Confrac, 28-VII-2007, *A. R. Burgaz*, MACB 97030; Balneario de Panticosa, 5-IX-1991, *T. Ahti, A. R. Burgaz & E. Fuertes*, MACB 44236; Bielsa, valle de Pineta, río Cinca, 25-VIII-2008, *A. R. Burgaz*, MACB 102813; Biescas y Yesero, valle de Asieso, 10-IX-1994, *J. Etayo, A. Gómez-Bolea & al.*, MA-lichen 6316; Biescas, Piedrafita de Jaca, 10-IX-1994, *A. R. Burgaz*, MACB 91000; Biescas, valle del Asieso, pinar-abetal, 9-IX-1994, *A. R. Burgaz & I. Martínez*, MACB 96660; Formigal, Barranco de Bronhuso, 6-IX-1991, *T. Ahti, A. R. Burgaz & E. Fuertes*, MACB 44285; Gistain, valle de Gistau, 27-VII-2003, *A. R. Burgaz*, MACB 91063; Ibon de Brazato, Panticosa, 7-IX-1994, *A. R. Burgaz*, MACB 90998, MACB 90999; Oza, subida al valle de Aguas Tuertas, valle de Hecho, 23-VII-2000, *A. R. Burgaz*, MACB 91114, MACB 91002; Panticosa, subida Ibón de Brazato, 7-IX-1994, *A. R. Burgaz*, MACB 102794; Salinas, valle de Gistau, 27-VII-2003, *A. R. Burgaz*, MACB 91062; Saravillo, hacia refugio Labasar, valle de Gistau, 27-VII-2003, *A. R. Burgaz*, MACB 91061; Torla, Ordesa, Río Arazas, cascada Torconelbotera, 19-VII-1993, *I. Martínez*, MACB 90990; Jaén: Albanchez de Mágina, Puerto Albanchez, Sª de Mágina, 6-XII-2006, *A. R. Burgaz*, MACB 94351; Génave, 27-III-2003, *A. R. Burgaz*, MACB 91106; Segura de la Sª, alrrededores de El Yelmo, 22-II-2002, *A. R. Burgaz*, MACB 102796; La Coruña: Betanzos, Espenuca, 22-X-1994, *A. R. Burgaz*, MACB 91020; San Andrés de Teixido, 23-X-1994, *A. R. Burgaz*, MACB 90993, MACB 91019; La Rioja: Ajamil, Sª de Camero Viejo, 20-IX-1990, *A. R. Burgaz & E. Fuertes*, MACB 44249; Canales de la Sª, 19-IX-1990, *A. R. Burgaz & E. Fuertes*, MACB 44254; Ezcaray, Posadas, 30-VIII-1996, *A. R. Burgaz*, MACB 91042; Lumbreras, 21-X-1983, *A. R. Burgaz & Mendiola*, MACB 14665; Lumbreras, 22-X-1983, *A. R. Burgaz & Mendiola*, MACB 37212, MACB 14664; Mansilla de la Sª, 8-IX-2004, *A. R. Burgaz*, MACB 89736; Monterrubio, vertiente S de la Sª de la Demanda, 19-IX-1990, *A. R. Burgaz & E. Fuertes*, MACB 44250, MACB 44253; Posadas, Sª de San Lorenzo, valle del río Oja, 7-IX-2004, *A. R. Burgaz*, MACB 89520, MACB 89519; León: Brazuelo, Montes de León, 20-VII-2006, *A. R. Burgaz*, MACB 93518; Cerredo, 29-X-1994, *A. R. Burgaz*, MACB 92591; Cofiñal, pinar de Lillo, 6-VIII-2003, *A. R. Burgaz*, MACB 91033, MACB 91055; Colinas del Campo de Martín Moro, 21-X-1994, *A. R. Burgaz*, MACB 91047, MACB 90992; Manzanares del Puerto, 3-IX-2002, *A. R. Burgaz*, MACB 91081; Puerto de las Señales, 6-VIII-2003, *A. R. Burgaz*, MACB 91058; Puerto de San Isidro, 5-VIII-2003, *A. R. Burgaz*, MACB 91057; Tejeda de Ancares, 11-VII-1984, *A. R. Burgaz & E. Fuertes*, MACB 44234, MACB 44252; Vega de Espinareda, valle del río Burbia, 13-VIII-1994, *I. Martínez*, MACB 64876; Lérida: Barruera, subida a collado de Dellni, 10-VII-1994, *G. Aragón, J. Castillo, A. Herrero & I. Martínez*, MACB 90997; Espot, pista de Lladres, Rio de Peguera, 12-VII-1994, *G. Aragón, J. Castillo, A. Herrero & I. Martínez*, MACB 90994; Espot, Vall d'Espot, 13-VII-1994, *G. Aragón, J. Castillo, A. Herrero & I. Martínez*, MACB 90995; Espot, subida al lago de San Mauricio, 27-VII-1998, *A. R. Burgaz & I. Martínez*, MACB 94215; Montella, 2-VII-1996, *A. R. Burgaz & I. Martínez*, MACB 91091; Montellá, Sª Cadí, subida Prat d'Aguiló, 2-VII-1996, *A. R. Burgaz & I. Martínez*, MACB 90988, MACB 91039; Valencia d'Aneu, 7-VII-1994, *G. Aragón, J. Castillo, A. Herrero & I. Martínez*, MACB 90996; Valle de Arán, 21-VIII-1993, *I. Martínez*, MACB 90991; Viella, Hospital de Viella, 11-X-1996, *D. Manso & N. Marcos*, MACB 92846, MACB 91101; Viella, Tredos, río Aiguamotx, 11-X-1996, *D. Manso & N. Marcos*, MACB 91113; Lugo: Baleira, Alto de la Fontaneira, 20-VII-2006, *A. R. Burgaz*, MACB 93514; Gundriz, Municipio Samos, valle de Lauzara, 24-III-2005, *A. Noguerol Seoane*, MACB 98157, MACB 92560, MACB 92559; Queixoiro, arroyo invernal, 21-VII-2006, *A. R. Burgaz*, MACB 93150; S. Martín de Suarna, 21-VII-2006, *A. R. Burgaz*, MACB 93113; Madrid: Buitrago de Lozoya, 26-XI-1989, *A. R. Burgaz*, MACB 37188; Bustarviejo, Puerto de Canencia, 14-II-2000, *A. R. Burgaz*, MACB 97264; El Berrueco, 9-XI-2007, *A. R. Burgaz*, MACB 96005; El Cuadrón, 21-VI-1997, *A. R. Burgaz & S. Casas*, MACB 75218; Embalse de Riosequillo, 21-III-1997, *A. R. Burgaz & S. Casas*, MACB 75237; Hoyo de Manzanares, 1-XI-1989, *A. R. Burgaz*, MACB 37189; Monte de El Pardo, 9-IV-2004, *A. R. Burgaz*, MACB 91031, MACB 90987, MACB 92569; Montejo de la Sª, 18-VII-2002, *A. R. Burgaz*, MACB 91006; Montejo de la Sª, 26-X-2002, *A. R. Burgaz*, MACB 91008; Puerto de Canencia, 14-XI-1995, *A. R. Burgaz*, MACB 91003; Puerto de Canencia, 22-IV-1996, *A. R. Burgaz*, MACB 91007; Puerto de Canencia, 30-X-1991, *A. R. Burgaz*, MACB 44255; SomoSª, arroyo de la Peña del Chorro, 9-XI-2007, *A. R. Burgaz*, MACB 98075; Torrelaguna, 21-III-1997, *A. R. Burgaz & S. Casas*, MACB 75255; Málaga: Estepona, 17-III-1995, *G. Aragón & I. Martínez*, MACB 92562; Estepona, Sª Bermeja, Los Reales, 17-III-1995, *G. Aragón & I. Martínez*, MACB 91025; Jimena de la Frontera, P. Nat. de los Alcornocales, Sª del Algibe, La Sauceda, Garganta de Pasadallana, 27-IV-2001, *A. R. Burgaz, M. Carrasco & E. Fuertes*, MACB 91010; Navarra: Abázuza, pista del Montasterio de Iranzu, 12-X-1993, *J. Etayo*, MA-lichen 4410; Eugui, 8-IX-1991, *T. Ahti, A. R. Burgaz & E. Fuertes*, MACB 44239; Irati, Orbaiceta, 7-IX-1991, *A. R. Burgaz & E. Fuertes*, MACB 44237, MACB 44240; Lanz, 1-IX-1994, *J. Etayo*, MA-lichen 6546; Oronoz-Mugaire, señorio de Bértiz, subida a Aizcolegi por carretera, 15-XII-2001, *J. Etayo*, MA-lichen 13331; Puerto de Ibañeta, 77-IX-1991, *T. Ahti, A. R. Burgaz & E.*

Fuertes, MACB 44238; Tafalla, monte Plano, 20-V-1993, *J. Etayo*, MA-lichen 4165; Valle del Salazar, Usún, foz de Arbayún, cauce del río Salazar, 22-VIII-1994, *J. Etayo & W. Müller*, MA-lichen 5900; Orense: Cabeza de Manzaneda, Manzaneda, Sª de Queixa, 24-VIII-2002, *A. R. Burgaz*, MACB 91085; Subida a Cabeza de Manzaneda, Manzaneda, Sª de Queixa, 24-VIII-2002, *A. R. Burgaz*, MACB 91001, MACB 91089, MACB 91086; Villarino de Conso, Montes de Invernadeiro, 19-VI-1995, *A. R. Burgaz, I. Martínez & P. Navarro*, MACB 96661, MACB 96711; Palencia: Camporredondo de Alba, Sª de Otero, 18-VII-2004, *A. R. Burgaz*, MACB 91029, MACB 91030; Cardaño de Arriba, Sª de Alba, 18-VII-2004, *A. R. Burgaz*, MACB 91021, MACB 92561; Pozo de Curavacas, 30-VI-1996, *N. Marcos & P. Navarro*, MACB 91023; Resoba, Reserva Nacional de Fuentes Carrionas, 24-VII-2006, *A. R. Burgaz*, MACB 93516; Vidrieros, Reserva Nacional de Fuentes Carrionas, pozo del Curavacas, 23-VII-2006, *A. R. Burgaz*, MACB 93515; Pontevedra: Isla de Ons, 27-VIII-2002, *A. R. Burgaz*, MACB 91096; La Guardia, Monte Santa Tecla, 29-VIII-2002, *A. R. Burgaz*, MACB 91013; Marín, lago Castiñeira, 31-VIII-2002, *A. R. Burgaz*, MACB 91070; Moaña, Mirador de Faro Domaio, 1-IX-2002, *A. R. Burgaz*, MACB 91090; Salamanca: Beleña, 5-XI-2005, *A. R. Burgaz*, MACB 92850; Carpio de Azaba, 1-V-2007, *A. R. Burgaz*, MACB 95485; El Cabaco, Sª de la Peña de Francia, 6-XI-2005, *A. R. Burgaz*, MACB 92852, MACB 92851, MACB 92853; Frades de la Sierra, Sª de Frades, 5-XI-2005, *A. R. Burgaz*, MACB 92849; La Alberca, Sª de las Mestas, Puerto del Portillo, 6-XI-2005, *A. R. Burgaz*, MACB 92856; La Alberca, Sª de las Mestas, valle del río Batuecas, 6-XI-2005, *A. R. Burgaz*, MACB 92855, MACB 92859, MACB 92857; Monsagro, Sª de la Peña de Francia, Paso de los Lobos, 6-XI-2006, *A. R. Burgaz*, MACB 92854, MACB 92963; Peña de Francia, 26-IX-1991, *A. R. Burgaz & E. Fuertes*, MACB 44242; Valdemierque, 5-XI-2005, *A. R. Burgaz*, MACB 92847, MACB 92848; Segovia: Aguila Fuente, 14-XI-1993, *A. R. Burgaz*, MACB 91059, MACB 102827; Coca, Finca El Sesquero, 20-VII-2006, *A. R. Burgaz*, MACB 93736; El Espinar, valle del río Moros, área recreativa la Panera, 26-I-2008, *A. R. Burgaz*, MACB 96802, MACB 96803; La Granja, Valsaín, río Eresma, fuente de los Dos Caños, 26-I-2008, *A. R. Burgaz*, MACB 96805; La Pinilla, 6-VIII-2002, *A. R. Burgaz*, MACB 91024; Puerto de la Quesera, 2-VI-1995, *N. Marcos & Navarro*, MACB 91098; Puerto de Navacerrada, bajada de las 7 revueltas, 15-IV-1994, *A. R. Burgaz*, MACB 91097; Revenga, cordel de Peñas Zamarriegas, 26-I-2008, *A. R. Burgaz*, MACB 96804; Riofrio de Riaza, Puerto de la Quesera, 29-VI-1991, *A. R. Burgaz*, MACB 44251; Soria: Berlanga de Duero, 13-IV-2006, *R. Pino-Bodas*, MACB 96006; Laguna Negra, Sª de Urbión, 7-IX-2003, *A. R. Burgaz*, MACB 91069; Muriel Viejo, 12-VIII-2008, *A. R. Burgaz*, MACB 102894; Puerto de Santa Inés, 7-IX-2003, *A. R. Burgaz*, MACB 91079, MACB 91074, MACB 91051; Vinuesa, 7-IX-2003, *A. R. Burgaz*, MACB 91078; Tarragona: Cardó, Sª de Cardó, 9-IV-2003, *A. R. Burgaz*, MACB 91099; Conca de Barberá, Poblet, cuarcitas, *A. R. Burgaz*, MACB 102830; Conca de Barberá, Prades, 22-V-1998, *A. R. Burgaz*, MACB 91100; Refagueri, P. Nat. Els Ports, 9-IV-2003, *A. R. Burgaz*, MACB 91064, MACB 102810; Roquetes, bajada del Mont Caro, 10-IV-2003, *A. R. Burgaz*, MACB 102817; Teruel: Fonfría, Sª del Cucalón, 23-VI-1992, *A. R. Burgaz*, MACB 91109; Consuegra, Sª de Valdehierro, 5-XI-2004, *A. R. Burgaz*, MACB 91117; Sartajada, arroyo del Herrerillo, valle del Tietar, 14-IV-2002, *P. Aguilar, G. Amo & A. R. Burgaz*, MACB 97322; Urda, Sª Morrones, Finca El Convento, 5-XI-2004, *A. R. Burgaz*, MACB 91119; Urda, Sª Morrones, subida antena TV, 5-XI-2004, *A. R. Burgaz*, MACB 91116; Valladolid: Pedrejas de San Esteban, 20-VII-2006, *A. R. Burgaz*, MACB 93517, MACB 93738; Ureña, 20-VII-2006, *A. R. Burgaz*, MACB 93737, MACB 93745; Zamora: La Tabla, 6-X-1997, *G. Aragón, A. R. Burgaz & A. Terrón*, MACB 70178; Ribadelago, subida Pico del Fraile, P. Nat. Lago de Sanabria, 9-IX-1998, *A. R. Burgaz, S. Casas & I. Rodríguez de Lope*, MACB 70179; Sotillo de Sanabria, subida arroyo de las Truchas, P. Nat. Lago de Sanabria, 8-IX-1998, *A. R. Burgaz & S. Casas, I. Rodríguez de Lope*, MACB 70180; Zaragoza: Circo de San Miguel, Sª del Moncayo, 13-IX-2003, *A. R. Burgaz*, MACB 91054; Sestrica, 20-IX-2003, *A. R. Burgaz*, MACB 91052; Sestrica, suelo ácido, *A. R. Burgaz*, MACB 102826; Sª de Peña, puerto de Sos camino de Sos a Sádaba, 26-II-1998, *J. Etayo*, MA-lichen 13330; Tarazona, Sª de Moncayo, Barranco de Castilla, 4-IX-1984, *A. R. Burgaz*, MACB 44246, MACB 44245; Tarazona, Sª de Moncayo, Pico de San Miguel, 6-IX-1984, *A. R. Burgaz*, MACB 44244; Baleares Islands: Mallorca: A 5 Km de Palmanova y a 1 Km de playa del Mago, 12-VII-1994, *J. Etayo*, MA-lichen 6690; Algaida, camí vell d'Algaida, 10-I-2006, *A. R. Burgaz*, MACB 102833, MACB 102812; Felanitx, Santuario de San Sanlvador, 12-I-2006, *A. R. Burgaz*, MACB 92761; Llucmajor, 12-I-2006, *A. R. Burgaz*, MACB 102811; Llucmajor, Cala Blava, 12-I-2006, *A. R. Burgaz*, MACB 102809; Llucmajor, Cap Blanc, 12-I-2006, *A. R. Burgaz*, MACB 102806; Pollença, El Mal Pas, 11-I-2006, *A. R. Burgaz*, MACB 92766, MACB 92767; Sa Pobla, 11-I-2006, *A. R. Burgaz*, MACB 102801, MACB 92764; Sa Pobla, 11-I-2006, *A. R. Burgaz*, MACB 92765, MACB 102829, MACB 102828; Santa Margarita, 10-I-2006, *A. R. Burgaz*, MACB 102816; Santa Margarita, Son Serra de Marina, 10-I-2006, *A. R. Burgaz*, MACB 102795, MACB 92762, MACB 92763; Son Caulelles, Marratxi, VII-1985, *M. A. Font*, MACB 39101; Menorca: Alaior, finca al margen izquierdo en la carretera Maó-Fornells, km 15.5, pasado el cruce Alaior-Na Macaret, 30-XI-2007, *A. R. Burgaz*, MACB 96260; Alaior, Son Bou, camino a Torre Solí Nou, 29-XI-2007, *A. R. Burgaz*, MACB 96257; Ciudadella, alrrededores de la Naveta de Tudons, 1-XII-2007, *A. R. Burgaz*, MACB 96262; Ciudadella, Cala en Turqueta, 1-XII-2007, *A. R. Burgaz*, MACB 96263; Ciudadella, cala Galdana hacia cala Macarella, 29-XI-2007, *A. R. Burgaz*, MACB 96255; Ciudadella, Santandria, Reserva Natural, 28-XI-2007, *A. R. Burgaz*, MACB 96253; Ciudadella, Son Saura, 1-XII-2007, *A. R. Burgaz*, MACB 96264; Es Mercadal, monte El Toro, 28-XI-2007, *A. R. Burgaz*, MACB 96254; Es Mercadal, subida al castillo de Santa Agueda, 1-XII-2007, *A. R. Burgaz*, MACB 96261; Ferreries, camino hacia Ets Alocs, 29-XI-2007, *A. R. Burgaz*, MACB 96256; Maó, cala Mesquida, 30-XI-2007, *A. R. Burgaz*, MACB 96258; Maó, Es Grau, P. Nat. Es Grau, 30-XI-2007, *A. R. Burgaz*, MACB 96259; Canary Island: Tenerife, La Orotava, Aquamansa, La Caldera, 13-IV-2000, *H. Väre*, H; Isla de la Gomera, P. Nac. de Garajonay, Degollada Blanca, entre Igualero y casa forestal de Las Tajoras, XII-2002, *C. Hernandez-Padrón & P. L. Pérez de Paz*, MACB 94688; P. Nac. de Garajonay, Las Cancelas, 19-II-2002, *D. Sicilia*, MACB 94675; P. Nac. de Garajonay, carretera hacia San Sebastián, 19-II-2002, *D. Sicilia*, MACB 94676; P. Nac. de Garajonay, entre Fuensanta y el mirador de Vallehermoso, 19-II-2002, *D. Sicilia*, MACB 93485; Isla de la Gomera, P. Nac. de Garajonay, cerca del mirador de Vallehermoso, 15-IV-2000, *C. Hernandez-Padrón & D. Sicilia*, MACB 93484; Isla de la Gomera, P. Nac. de Garajonay, carretera hacia San Sebastián, 19-II-2002, *D. Sicilia*, MACB 93588; P. Nac. de Garajonay, Roque de La Zarcita, III-2002, *C. Hernadez-Padrón & P. L. Pérez de Paz*, MACB 94689; P. Nac. de Garajonay, cumbre del Carbonero, 19-III-2002, *C. Hernandez-Padrón, P. L. Pérez de Paz, D. Sicilia & I. P. Vargas*, MACB 93487; Agulo, Garajonay, 28-IV-1999, *A. Herrero*, MACB 97904; Valle Gran Rey, 29-VI-1999, *A. Herrero*, MACB 97906; Hermigua, 15-VIII-1999, *A. Herrero*, MACB 97905; San Sebastián de la Gomera, Bailadero, orientación NE, 4-V-1999, *A. Herrero*, MACB 97903; Hemigua, Tajaque, cerca del mirador (a 200 m), orientación norte, 4-VIII-1994, *J. Etayo*, MA-lichen 6347; Isla de El Hierro, Frontera, P. Nat., 5-X-2001, *N. Marcos & D.Manso*, MACB 91082; Isla de Tenerife, las Mercedes, Monte de las Mercedes, hacia Batones, 16-VI-2007, *A. R. Burgaz*, MACB 97907; La Orotava, subida al Teide, 15-VII-2007, *A. R. Burgaz*, MACB 97901, MACB 97902; La Orotava, pinar de Aguamansa, 29-IV-1935, MACB 9891; **Sweden**: Södermanland: rödinge sn, Norrga, VSV om Atorp. Pa marken bland mossa i barrskog, 19-III-1997, *G. Odelvik*, S L10807; Västmanland: Guldsmedhytte par., Näset. Bladmossa och gräs på en gråalbevuxen dikesren, IV-1933, *S. Schiöler*, UPS L-73894; Guldsmedhytte par., Ustaboda. Stenróse vid sjöstraden, X-1935, *S. Schiöler*, UPS L-73890; Sala SV om Sala kyrka, Sala gruva, Gruvbyn, barrblandskig med ädellövinslag, pa sten., 17-X-2001, *C. Hammarberg*, S L27408; **Switzerland**: Neuchâtel: Boven Lac des Taillières, 17-IV-1966, *J. H. Wieffering*, L 794565; **Thailand**: Doi Inthanon National Park, 31-III-005, *S. Pommen*, H; Northeasttheon Thailand, Phu leanmy willdlife center, 3-IX-

2005, *S. Pommen*, H; **Turkey**: Giresum: Kesap, Degirmenagzi village, 12-II-2006, *K. Kinalioglu*, H; Trabzon: Arakli, S of Kizilkaya Yoylasi, 18-VIII-2005, *K. Kinalioglu*, H; Zonguldak: Beycuma County, Cayköy district and surrounding, 20-VIII-2005, *Y. Yazici*, H; Beycuma County, Cayköy district and surroundings, 20-VIII-2005, *K. Kinalioglu*, H; **United Kingdom**: Scotland: Altnafeadh, valle de Glencoe, Highland District, 12-VI-2002, *A. R. Burgaz*, MACB 97900; Dallwhinmie, Highland District, 11-VI-2002, *A. R. Burgaz*, MACB 97899; Wales: South Wales 0.5 mile S of Majors Creek, Tallaganda Shire, S. Tablelands, 18-X-1967, *L. G. Adams*, L 794562; **United States**: Alaska: Hogg Is., Kodiak, 4-VII-2008, *K. Dillman*, H; Arkansas: Polk County, Caney Creek Wilderness of Ouachita National Forest. East slope of Buckeye Mt. near old mine, east facing hillside with large boulders in oak-hickory, 21-V-2000, *C. M. Wetmore*, S L12247; Polk County, Caney Creek Wilderness of Ouachita National Forest, east slope of Buckeye Mt. near old mine. East facing hillcing hillside with large boulders in oak-hickory, 21-V-2000, *C. M. Wetmore*, CANB 568690; Arkansas: Polk Co, Caney Wilderness of Ouchita National Forest. East slope of Buckeye Mt. Near old mine, 21-V-2000, *C. M. Wetmore*, UPS L-108510; California: Marin County, Greek and along trail to Redwood Grove picnic Area, 4-VI-2002, *C. Lendemer*, FH; Florida: Cedar Crek area, 29 Km northeast of Ocala Florida, north of Highway, 21-XII-2001, *R. Rosentreter*, FH; Illinois: Peoria county, near Peoria, at Rocky Glen, atop sandstone ravine, 30-III-008, *R. D. Hyerczyk*, F; New Hampshire: Carrol County, Moultoboro, Ossipee Mountains, Bald Knob, 15-IX-2007, *M. Schmull*, FH 259363; New York: Tompkins County, town of Dryden, cornell University's McLean Bogs Reserve W of Cortland County Line, S of Groton Town line, 26-I-2002, *H. T. Root*, FH; North Carolina: Macon Co. Adjacent to Lake Ravenel and Highlands Biological Stations Botanical Garden, Highlands Biological Stations east of Highlands, 12-VII-2002, *J. C. Lendemer*, UPS L-129919; Macon County, in near full shade, adjacent to Lake Ravenel and Highlands Biological Station Botanical Garden, Highland Biologicao Station, east of Higland, 12-VII-2002, *J. C. Lendemer*, CANB 671668; Transylvania County, Pilot Mountaint, Art Loeb Trail, north from Glaucester gap, 1-II-2003, *E. Tripp & D. Clarke*, FH; Ohio: Adams county, Edge of Appalachia Nature Preserve, Buzzardroost Rock Trail, Brush Creek, 20-V-006, *R. D. Hyerczyk*, F; Pennsylvania: Berks County, northeast side of Hawk Mountain, 100 yard below the entrance to the Rover of Rocks Trail, Hawk Mountain Sanctuary, 10-VI-2002, *J. C. Lendemer*, FH; Luzerne County, 27-III-2004, *J. C. Lendemer*, FH; Luzerne County, 27-III-2004, *J. C. Lendemer*, FH; Virginia: West Virginia, Pocahontas County, Monogahela National Forest, 0-1 mile south of intersection of FS road and CR 23, west slope of Middle Mountain, vicinity of Devil's Garden, Lake Sherwood Quad., 21-X-2007, *J. C. Lendemer*, FH 239444; Washington: Bald/Hill near Deschetes Falls, 39 milles S. E. of Olympia, 15-VIII-1953, *Coll & Det. A. W. C. T. Herre*, L 794561; **Uruguay**: Lavalleja: Waterfall of Penitente brook, 9-IV-2001, *S. Hammer*, CANB 579168; **Unknown**: 27-V-1910, *G. K. Merill*, L 794559.

***Cladonia glauca* Flörke**

Czech Republic: Karlovy Vary: Slavkovský les upland, Sokolov, Horni Slavkov, quarry "Jáma Hubert" c. 1.5 Km S of town, 18-IV-2009, *J. Vondrák* 7023; **Denmark**: Hillerod, Tisvildeleje, Woodland area Tisvilde SW of town, 14-III-2009, *J. Vondrák* 6966; **Spain**: Álava: Tertanga, Puerto de Orduña, 25-VII-2006, *A. R. Burgaz*, MACB 96090; Asturias: Pajares, puerto de Pajares, 22-VII-2007, *A. R. Burgaz*, MACB 95508; Navarra: Lanz, 1-IX-1994, *J. Etayo*, MA-lichen 6522; Salamanca: El Cabaco, Sª de la Peña de Francia, 6-XI-2005, *A. R. Burgaz*, MACB 96579; Segovia: La Granja, Valsaín, río Eresma, fuente de los Dos Caños, 26-I-2008, A. R. Burgaz, MACB 96751.

***Cladonia gracilis* subsp. *gracilis* (L.) Willd.**

Andorra: Soldeu, Port d'Envalira, 19-VIII-2006, *A. R. Burgaz*, MACB 94196; **Argentina**: Tierra de Fuego: Ruta Nacional 3, desde Tolhuin a Las Termas, 15-III-2005, *E. Fuertes*, MA-lichen 16116; **Austria**: Steiermark: Seetaler Alpen, 10 Km WNW of Obdach, road from Schmelz to Winterleitenhütte, 28-V-2003, *W. Obermayer*, UPS L-135110; **Chile**: Región XII, Magallanes y Antártica Chilena: Isla de Navarino, Lago Róbalo, 18-I-2005, *R. Vilches*, MACB 92205; Isla de Navarino, camino de Lum, 26-I-2005, *R. Vilches*, MACB 91964; Isla de Navarino, Puerto Williams, valle del río Ukika, camino a Media Luna, 18-I-2005, *A. R. Burgaz*, MACB 91963; Isla de Navarino, rocas supramareales del canal Murray, 19-I-2005, *J. Izaguirre & I. Suberbiola*, MACB 91962; Isla de Navarino, Dientes de Navarino, 19-I-2005, *Popi*, MACB 92146; Islas Holguer, frente a caleta Eugenia de Isla Navarino, 19-I-2005, *J. Izaguirre & I. Suberbiola*, MACB 91961; **Denmark**: Bornholm: Molleodde, 14-VI-1997, *E. S. Hansen*, H; Molleodde, 14-VI-1997, *E. S. Hansen*, H; Jutland: Hårup Sande E of Silkeborg, L 794574; Hanstedt Reservatet (ca. 15 Km NW of Thisted), 7-VIII-1979, *O. Vitikainen*, H; Jylland: Hulsing Hede, 30-X-003, *S. N. Christensen*, H; Kandestederne, 26-VI-2000, *E. S. Hansen*, H; Laeso, Holtemmen, 2-VII-1996, *S. N. Christensen*, H; Silkeborg, Vesterskoven, 5-X-1967, *R. Bäck*, H; Silkeborg, Vesterskoven, Kongshus, halmtak, 5-X-1967, *R. Bäck*, H; Zealand: Sjaelland, Dronning Molle, Rusiand, 8-XII-2004, *P. Corfixen, S. Christensen & E. S. Hansen*, H; **Finland**: Finland Proper: Immula, Sorronsolo Lohja rural municipality, 19-VI-1990, *T. Ahti, A. R. Burgaz E. Fuertes & Isoviita*, MACB 50774; Kymenlaakso: Hamina, Vehkalahti, Pyhältö, 12-IX-2008, *V. Haikonen*, H; Päijänne Tavastia: Asikkala, Myllykselä, Huuhinsaari, 7-III-2004, *V. Haikonen*, H; Asikkala, Revilä, Paljumäki, 20-X-2002, *V. Haikonen*, H; Asikkala, Rutalahti, Vaajankallio, 8-VI-2009, *V. Haikonen*, H; Hämeenkoski, Miehola, Mustikkamäki, 22-V-2003, *V. Haikonen*, H; Hämeenlinna, Lammi, Kuohijärvi, Hietasalo, 20-III-2009, *V. Haikonen*, H; Hartola, Jääjärvi, Kuukivensaaret, 18-III-2009, *V. Haikonen*, H; Heinola, Mataraniemi, 31-VIII-2008, *V. Haikonen*, H; Heinola, Vanhakylä, Pässivuori, 14-VI-2008, *V. Haikonen*, H; Hollola, Hankaa, Rautakorpi, 12-IV-2008, *V. Haikonen*, H; Jaala, Kimola, Taivaljärvenvuori, 17-IV-2008, *V. Haikonen*, H; Kärkölä, Marttila, Pekkalankalliot, 28-VI-2007, *V. Haikonen*, H; Kuhmoinen, Harmoinen, Huhkaimenvuori, 15-V-2008, *V. Haikonen*, H; Kuhmoinen, Kotakoski, Kotavuori, 30-VII-2008, *V. Haikonen*, H; Nastola, Ruuhijärvi, 3-XI-2003, *V. Haikonen*, H; Nastola, Seesta, 13-X-2002, *V. Haikonen*, H; Sysmä, Vehkasalo, Huhkaimenvuori, 5-X-2008, *V. Haikonen*, H; Tuulos, Multasilta, 24-IV-2003, *V. Haikonen*, H; Pirkanmaa: Kangasala, Kuohenmaa, Nälkäkallio, 19-XI-2000, *M. Kääntönen*, H; Orivesti, Rajalahti, Tuomisto, 12-X-2002, *M. Kääntönen*, H; Tampere, Teisko, Kapee, Kulhanvuori, 24-X-1998, *M. Kääntönen*, H; Southern Savonia: Enonkoski, Haponniemi, 23-V-2006, *V. Haikonen*, H; Tavastia Proper: Kalvola, Könnölä, Kaivolampi, 18-IV-2008, *H. Väre*, H; Kalvola, Könnölä, Kaivolampi, 18-IV-2008, *H. Väre*, H; Kalvola, Könnölä, Kaivolampi, 18-IV-2008, *H. Väre*, H; Kalvola, Könnölä, Kaivolampi, 18-IV-2008, *H. Väre*, H; Uusimaa: Anjalankoski, Junkkari, 20-IV-2006, *V. Haikonen*, H; Artjärvi, Hiitelä, Pulikankallio, 9-IV-2003, *V. Haikonen*, H; Askola, Juornaankylä, Kirkeakallio, 15-IV-2003, *V. Haikonen*, H; Askola, Särkijarvi, Falkviikinkallio, 10-XI-2003, *V. Haikonen*, H; Elimäki, Mustila, 25-V-2005, *V. Haikonen*, H; Myrskylä, Kreivilä, Laukkallio, 5-V-2007, *V. Haikonen*, H; Orimattila, Heinämaa, Porttikallio, 8-XI-2002, *V. Haikonen*, H; Orimattila, Leitsamaa, Porttikallio, 25-XI-2005, *V. Haikonen*, H; Orimattila, Pyörähtälä, Hannunkallio, 7-VIII-2008, *V. Haikonen*, H; Pernaja, Tervik säteri, 11-IV-2009, *V. Haikonen*, H; Pornainen, Vähä-Laukkoski, 10-V-2008, *V. Haikonen*, H; Pukkila, Haarajoki, Venunmetsä, 8-X-2008, *V. Haikonen*, H; Sipoo, Talma, Byända, E. of Tallbacka, 18-IX-1980, *O. Vitikainen*, MACB 64956; **France**: Auvergne: Central Albepierre near Murat, 26-VII-1998, *L. Spier*, L 753024; **Iceland**: At Naumimelur, Fannarlur, Nordfjördur, near the river

Nordfjardara and the lake Naumamelstjörn, 28-VII-1991, *P. Uotila & A. Kurtto*, H; **Latvia**: Tukums: 5 Km N of Engure Fild starion on W shore of Lake Engure, 31-VIII-1988, *P. Uotila*, H; **New Zealand**: South Island: Mt. Cook area, above Hermitage, II-1974, *B. O. van Zanten*, L 794570; **Norway**: C.W. Greenland: Disko, Nordfjor, 11-VIII-1975, *V. Alstrup*, MA-lichen 5276; Nord-Norge: Finnmark, Nesseby, Mortensnes, 22-VIII-1974, *T. Ahti & O. Vitikainen*, H; Tromso: Lyngen, Lyngseidet, 12-VIII-1959, *T. Ahti & F. Skuncke*, H; **Poland**: Lesser Poland: Cracow, Artic, Svalbard, Spitsbergen, Bellsund, Chamberlindalen, VIII-1988, *F. Swies*, H; **Portugal**: Beira Alta: Estrela, Sª da Estrela, Ribeira da Pragueira, 22-X-1995, *N. Marcos, P. Navarro & E. Munín*, MACB 66846; La Torre, Sª da Estrela, P. Nat. de Sª da Estrela, 3-V-2007, *A. R. Burgaz*, MACB 94921; Manteigas, Sª da Estrela, P. Nat. de Sª da Estrela, poço do Inferno, 2-V-2007, *A. R. Burgaz*, MACB 94024; Penhas da Saúde, Sª da Estrela, P. Nat. de Sª da Estrela, 2-V-2007, *A. R. Burgaz*, MACB 95309; Penhas Douradas, Sª da Estrela, P. Nat. de Sª da Estrela, 2-V-2007, *A. R. Burgaz*, MACB 95275, MACB 94925, MACB 94922; Sabugueiro, Lagoa Comprida, Sª da Estrela, P. Nat. de Sª da Estrela, 3-V-2007, *A. R. Burgaz*, MACB 94923; Saburgueiro, Lagoa Compridda, Sª da Estrela, P. Nat. de Sª da Estrela, 3-V-2007, *A. R. Burgaz*, MACB 94926; Beira Litoral: Sª do Açor, Fraga de Pena Barroco de Degrainhos, 1,5 Km de Benfeite, 29-III-1998, *T. Ahti & A. R. Burgaz*, MACB 66844; Minho: Serra Amarela, Ermida, 16-VI-1995, *A. R. Burgaz, I. Martínez & P. Navarro*, MACB 66845; Trás-Os-Montes: Montezinho, Sª de Montezinho, 8-IX-2006, *R. Pino*, MACB 93693; Lagoa, valle del río Sabor, 5-IX-2006, *R. Pino-Bodas*, MACB 93691; Lagoa, valle del río Sabor, 5-IX-2006, *A. R. Burgaz*, MACB 93866; Montezinho, Sª de Montezinho, 8-IX-2006, *A. R. Burgaz*, MACB 94133, MACB 93867; Nogueira, Sª de Nogueira, 20-II-2005, *A. R. Burgaz*, MACB 94218; **Russia**: Leningrad: Karelia australis, Ka/Rs, Vyborg District, Berëzovye Island reserve, Bol'shoy Berëzovyy Island Poles'e, 12-VIII-2008, *T. Ahti*, H; Western Leningrad region, 1 Km NW of Konmovo village, 14-IV-2007, *D. Himelbrant*, H; **Spain**: Asturias: Vega de Espinareda, valle del río Burbia, 13-VIII-1994, *G. Aragón & I. Martínez*, MACB 95192; Ávila: Peguerinos, alrrededores del campañento "Peñas Blancas", 27-X-1996, *A. R. Burgaz*, MACB 95189; Burgos: Huidobro, 7-VI-1988, *A. R. Burgaz*, MACB 44577; Neila, Sª de Neila, 6-IX-2003, *A. R. Burgaz*, MACB 95190; Pineda de la Sª, Sª de Mencilla, 13-III-2008, *A. R. Burgaz*, MACB 97631; Villasur de Herreros, Sª de San Millán, valle del río Arlanzón, 13-III-2008, *A. R. Burgaz*, MACB 97611; Villasur de Herreros, Sª de San Millán, valle del río Arlazón, 13-III-2008, *A. R. Burgaz*, MACB 97630; Cáceres: NavatraSª, Sª de la Palomera, 5-V-2007, *A. R. Burgaz*, MACB 94920; San Martín de Trevejo, Sª de Gata, río de la Vega, 4-IV-1996, *G. Aragón, A. Herrero & I. Martínez*, MACB 95116; Cantabria: Peña Sagra, 10-VII-1985, *A. R. Burgaz*, MACB 44574; Guadalajara: Cantalojas, barranco del río Lillas, P. Nat. Tejera Negra, 23-VIII-2003, *A. R. Burgaz*, MACB 95195; Huesca: Astún, Valle de Canfrac, 28-VII-2000, *A. R. Burgaz*, MACB 96713; La Coruña: Villarpaso, Aranga, 3-III-1967, *J. Dalda*, MACB 2973; La Rioja: Canales de la Sª, 19-IX-1990, *A. R. Burgaz*, MACB 45267; Lumbreras, 21-X-1983, *A. R. Burgaz & Mendiola*, MACB 14656, MACB 37179; Mansilla de la Sª, 8-IX-2004, *A. R. Burgaz*, MACB 89567; León: Brazuelo, Montes de León, 20-VII-2006, *A. R. Burgaz*, MACB 95895; Brazuelo, Montes de León, 20-VII-2006, *A. R. Burgaz*, MACB 94439; Cacabelos, muros de caliza, 11-VII-1984, *J.M. Fuente*, MA-lichen 4055; Cofiñal, pinar de Lillo, 6-VIII-2003, *A. R. Burgaz*, MACB 95196, MACB 95186; Pico Cuiña, Puerto de Ancares, 12-VII-1984, *A. R. Burgaz*, MACB 44575; Puerto de las Señales, 6-VIII-2003, *A. R. Burgaz*, MACB 95197; Puerto de San Isidro, 5-VIII-2003, *A. R. Burgaz*, MACB 95191; Tejedo de Ancares, 11-VII-1984, *A. R. Burgaz*, MACB 44576; Lugo: Baleira, Alto de la Fontaneira, 20-VII-2006, *A. R. Burgaz*, MACB 95225; Fonfría, subida al Puerto del Acebo, Sª de Linares, 21-VII-2006, *A. R. Burgaz*, MACB 95162; Gundriz, Municipio de Samos, valle de Louzara, 24-III-2005, *A. Noguerol Seoane*, MACB 92570; Queixoiro, arroyo Invernal, 21-VII-2006, *A. R. Burgaz*, MACB 93243; Madrid: Miraflores de la Sª, puerto de Canencia, 14-II-2000, *A. R. Burgaz & I. Martínez*, MACB 95193; Navarra: Orbaiceta, hayedo de Irati, 7-IX-1991, *T. Ahti, A. R. Burgaz & E. Fuertes*, MACB 44578; Palencia: Camporredondo de Alba, Sª de Otero, 18-VII-2004, *A. R. Burgaz*, MACB 97564, MACB 94217; Cervera de Pisuerga, Reserva Nacional de Fuentes Carrionas, 20-III-1992, *I. Martínez*, MACB 44579; Vidrieros, Reserva Nacional de Fuentes Carrionas, Alto de Riofrío, 23-VII-2006, *A. R. Burgaz*, MACB 94440; Vidrieros, Reserva Nacional de Fuentes Carrionas, Alto de Riofrío, 23-VII-2006, *A. R. Burgaz*, MACB 95218; Vidrieros, Reserva Nacional de Fuentes Carrionas, Alto de Riofrío, 23-VII-2006, *A. R. Burgaz*, MACB 97629; Salamanca: La Alberca, Sª de la Peña de Francia, 10-V-2003, *G. Amo de Paz*, MACB 97196; Monsagro, Sª de la Peña de Francia, Paso de los Lobos, 6-XI-2005, *A. R. Burgaz*, MACB 94850; Monsagro, Sª de la Peña de Francia, Pasos de los Lobos, 6-XI-2005, *A. R. Burgaz*, MACB 94851; Peña de Francia, 26-IX-1991, *A. R. Burgaz*, MACB 44580; Segovia: La Granja, Valsaín, río Eresma, fuente de los Dos Caños, 26-I-2008, *A. R. Burgaz*, MACB 96712; Riofrío de Riaza, Puerto de la Quesera, 29-VI-1991, *A. R. Burgaz*, MACB 45266; Riofrío de Riaza, Puerto de la Quesera, 29-VI-1991, *A. R. Burgaz*, MACB 44572; Riofrío de Riaza, Puerto de la Quesera, 2-VI-1995, *A. R. Burgaz, I. Martínez & P. Navarro*, MACB 95188; Valsaín, Puerto de Navacerrada, 15-IV-1994, *A. R. Burgaz*, MACB 95187; Zamora: Porto, embalse de Porto, P. Nat. "Lago de Sanabria", 9-IX-1998, *A. R. Burgaz, S. Casas & I. Rofríguez de Lope*, MACB 70183; Porto, Laguna Sanabreza, P. Nat. "Lago de Sanabria", 9-IX-1998, *A. R. Burgaz, S. Casas & I. Rofríguez de Lope*, MACB 70182; Ribadelago, embalse de Garardones, P. Nat. "Lago de Sanabria", 9-IX-1998, *A. R. Burgaz, S. Casas & I. Rodríguez de Lope*, MACB 70184; Ribadelago, Sanabria, 6-IV-1989, *A. R. Burgaz*, MACB 95194; Ribadelago, subida Pico del Fraile, P. Nat. "Lago de Sanabria", 9-IX-1998, *A. R. Burgaz, S. Casas & I. Rofríguez de Lope*, MACB 70185; Tarazma, Sª de Moncayo, 6-IX-1984, *A. R. Burgaz*, MACB 44573; **South Africa**: Houtenigua, *Van den Bosch*, L 794571; **Sweden**: Dalarna: 15 Km W of Hedemora, S facing slope of Bispberg, 13-IV-1979, *T. Goward*, H; Rättvik par., Tättviksheden, vid kalkverket, 4,1 Km ONO Rättvik, 22-IX-2000, *J. Hermansson*, UPS L-157806; Södermanland: St. Malm, Brännkärr, 1915, *O. Gust*, H; Uppland: Uppsala, Fiby nature reserve, 2-VI-1985, *R. Skytén*, H; Uppsala, Stadsskogen, N end, 17-IV-2005, *T. Ahti*, H; Västerbotten: Asele Lappmark, Vilhelmina, 10 Km E of Klimpfjäll, hill Röberget by lake Kultsjön, 7-VIII-1991, *T. Ahti*, H; **Switzerland**: Wallis: Wallis Arolla, Evolènse, 23-VII-1990, *L. Spier*, L 753026; **United Kingdom**: Scotland: Tioram, 27-VII-2004, *L. Spier*, L 753022.

***Cladonia gracilis* subsp. *elongata* (Wulfen) Vainio**

Argentina: Tierra de Fuego: Ruta Nacional 3, desde Tolhuin a Las Termas, 15-III-2005, *E. Fuertes*, MACB 93257; **Canada**: Newfoundland: Labrador, Goose Bay, 3-VI-1950, *J. M. Gillett & W. I. Findley*, H; St. Barbate South District, Gros Morne National Park, Bonne Bay, 10-VIII-1978, *T. Ahti*, H; **Chile**: Aisén: P. Nac. de Rio Simson, along route 245, 24-IX-1981, *H. Kashiwadani*, L 794568; Región XII, Magallanes y Antártica: Isla de Navarino, camino de Lum, 26-I-2005, *R. Vilches*, MACB 92150; Isla de Navarino, cerro de la Bandera, 15-I-2005, *A. R. Burgaz*, MACB 92149; Isla de Navarino, Lago Róbalo, 18-I-2005, *R. Vilches*, MACB 92148; Isla de Navarino, Dientes de Navarino, 19-I-2005, *Popi*, MACB 92147; Puerto Natales, río Serrano, 30-I-2005, *A. R. Burgaz*, MACB 92152; Puerto Natales, seno Última Esperanza, 30-I-2005, *A. R. Burgaz*, MACB 92151; **Finland**: Lapland: Inari Lapland, Inari, Kaamanen, Tuuruharju, 23-VIII-1974, *T. Ahti*, H; Inari Lapland, Inari, Laanila, 11-VIII-1933, *R. Kalliola*, H; Inari Lapland, Inari, Paadar, 2-VII-1960, *T. Ahti*, H; Inari Lapland, Inari, Raja-Jooseppi, 0.4 Km SW of Frontier Guard Station, 30-VI-1959, *T. Ahti*, H; Inari Lapland, Riutula, Kettukangas, Tirron tie, Kettujoen sillasta, 1-X-1967, *T. Ahti*, H; Inari Lapland, Utsjoki, 1 Km N of Karigasniemi, 2-VII-1960, *T. Ahti*, H; Inari Lapland, Utsjoki, Kevojoki valley ca., 2 Km SW of Kevo Reseach Station, 20-VIII-1965, *T. Ahti*, H; Inari Lapland, Utsjoki, Utsjoki village, on bank of Tana (Teno) River, 8-IX-1959, *T. Ahti*, H; KiL, Kittilä, Köngäs, 14 Km S of Tepasto, 29-VIII-1959, *T. Ahti*, H; Lapponia

Kemensis, Muonio Jerisjärvi, Keimiöniemi, 2-VII-1867, *J. P. Norrlin*, H; Lapponia Kemensis, Muonio Jerisjärvi, Keimiöniemi, 2-VII-1867, *J. P. Norrlin*, H; Sodankylä, Vuotso, Pyhätunturi (Nattaset), 4-VIII-1933, *R. Kalliola*, H; Sodankylä, Ylikitinen, Laiti, 1.5 Km SE of the mouth of Kuolpujoki, 26-VIII-1959, *T. Ahti*, H; Sodankylä, Ylikitinen, Laiti, 1.5 Km SE of the mouth of Kuolpujoki, 26-VIII-1959, *T. Ahti*, H; North Karelia: Pohjois-Karjala, Ilomantsi, Mekrijärvi, Mekrijärvi Biological Station, 13-VI-2003, *T. Ahti*, H; Päijänne Tavastia: Asikkala, Vesivehmaan lentokenttä, 7-IX-2007, *V. Haikonen*, H; Satakunta: Enintekiön Lappi, Enotekiö, Sonkamuotka, 1-IX-1959, *T. Ahti*, H; Par. Muonioniska, prope Kathesuanto, 1867, *J. P. Norrlin*, H; South Ostrobothnia: Kristinestad, Lappfjärd, hill Bötombergen, Jungfrudansen, 20-IX-1992, *T. Ahti & A. R. Burgaz*, MACB 50799; **Poland**: Lesser Poland: Cracow Arctic, Svalbard, Spitsbergen, Bellsund, Lyellstanda, SE part, NE botton of Wijkanderberget, VIII-1988, *F. Swies*, H; Cracow, Arctic, Svalbard, Spitsbergen, Hornsund, Kvartsittsletta, between Kvartsittodden and Andersenpynten, VIII-1988, *P. Osyczka*, H; **Russia**: Arkhangelsk Oblast: Franz Josef Land, Ziegler Island, north-westrm part, near SW coast "Vestibül", 30-VII-1996, *H. Pauli*, H; Gub. Archangelsk Ad flum, Peza, prope Lobanovskaya, 14-VI-1891, *A. O. Kihlman*, H; Irkutsk Oblast: North Siberian Lowland, Mouth of the Yenisey River, right bank, in the vicinity of the settlement Ust-Port, 24-VIII-1992, *T. N. Otnyukova*, H; Kranosyarsk: N of Central Siberia Severnaya Zemlya Archipelago, NW extremity of Bol'shevik Is., Ostantsovaia River at 2 Km above its mouth at Mikoyan Bay, 12-VII-1996, *M. Zhurbenko*, H; N of Central Siberia, Izvestii TsIK archipelago in the Kara Sea, southern coast of Sverdrup Is., 1-VIII-1992, *Y. Kozhevnikov*, H; N of Central Siberia, Severnaya Zemlya Archipelago, NW extremity fo Bol'shevik Is., peninsula with Cape Baranova, E coast of Shokal'skogo Strait, 1 Km SE of "Mys Baravona", 10-VII-1996, *M. Zhurbenko*, H; N of Central Siberia, Severnaya Zemlya Archipelago, NW extremity of Bol'skogo Strait, 0.5 Km SE of "Mys Baravona", 10-VII-1996, *M. Zhurbenko*, H; Kranosyarsk: N of central Siberia, Severnaya Zemlya Archipelago, southern coast of Bol'shevik Is. Opposite Cape Chelyuskin at Taimyr coast, 19-VII-2000, *N. Matveeva*, H; Krai: Middle Siberian Plateau, Putorama Plateau, 20-VIII-1992, *T. N. Otnyukova*, H; N of Central Siberia, center of Taimyr Peninsula, Byrranga Mts., mid section of Bol'shaya Bootankaga River, 10-VII-1991, *V. Kuvaev*, H; Murmansk: Karelia keretina, Murmansk Highway, 3 Km N of Karelian boundary, 4 Km NW of Poyakonda, 18-VII-1996, *T. Ahti*, H; Lapponia Imandrae, Imandra, 14-IX-1993, *H. Väre*, H; Lapponia Imandrae, Kirovsk district, in valley of river Kunijok, Kuelporr, 6-VII-1998, *K. Kärkkäinen*, H; Lapponia Imandrae, Kirovsk District, Khibiny Mts., in valley of river Partomjok, 9-VII-1998, *K. Kärkkäinen*, H; Lapponia Imandrae, Lapland Biosphere Reserve, Chunatndra, SE part, 20-VII-1996, *T. Ahti*, H; Murmanskaya Distr., Nature Reserve Pasvik, 29-VII-2008, *M. A. Fadeeva*, H; Murmanskaya Distr., Nature Reserve Pasvik, 30-VIII-2008, *M. A. Fadeeva*, H; Murmanskaya Distr., Nature Reserve Pasvik, 31-VII-2008, *M. A. Fadeeva*, H; Nenets: Northeasten part of the Malozemel'skaya tundra, 8-VII-1999, *O. Lavrinenko*, H; Northeastern part of the Malozemel'skaya tundra, Peschanka-To Lake, 21-VIII-1998, *O. Lavrinenko*, H; Sakha republic: Kobyay distric, Ust' Vilyuy Range (part of Verkhoyansk Mts.), on the northern tributary of Khoyguolakh River, 3-4-VII-2002, *T. Ahti*, H; Tyumen Oblast: Siberia, Tyumen Region, Polar Ural Mountains, middle course of Sob' River, by Station 126 of the railway, 13-VII-1986, *M. P. Zhurbenko*, H; Siberia, Tyumen region, Polar Ural Mountains, middle course of sob' River, by Station 126 of the railway Vorkuta, Labytnangi, 16-VII-1986, *M. P. Zhurbenko*, H; **Sweden**: Dalarna: Särna par., Fulufjällents nature reserve just N and S of thje path westwards from Njup-Skärs waterfall, *G. Thor*, UPS L-150623; Härjedalen: Vemdalen par., Oxsjövålen, 16-VII-1915, *G. T. Cedergen*, UPS L-144601; Jämtland: Åre par., Storlien, vid högfjällshollet, 24-VII-1950, *P. Lindhl*, UPS L-153348; Uppland: Danmark par., Linnés Hammarby, 2,4-2,5 Km SE of Damark church, the hill NE of the hauses "Hammeren", 15-IX-2007, *G. Thor*, UPS L-167619.

***Cladonia gracislis* subsp. *tenerrima* Ahti**

Australia: Australian Capital Territory: Mt Coree, Brindabella Range, 29 Km W of Camberra, 12-XI-1998, *H. Streimann*, CANB 604538; Mt Coree, Brindabella Range, 29 Km W of Camberra, 12-XI-1998, *H. Streimann*, H; New South Wales: Hedley Tarn, near Blue Lake, 7 Km NE of Mt Kosciusko, 5-XII-1979, *H. Streimann*, H; Southern tablelands, Tinderry Range, Southern foothills of Mt. Tinderry, near Michelago Captains Falt road 10 Km SE of Michelago, 27-IX-1975, *D. Verdon*, H; Tinderry Mountain, 10 Km ESE of Michelago, 15-XI-1981, *H. Streimann*, H; Queensland: S. Tablelands, Budawang Range, 8 Km NE of Nerriga, Morton National Park, 1-V-1982, *A. W. Archer*, H; Tasmania: Track to Marions Lookout, 30-XI-1983, *A. W. Archer*, H; Victoria: North Jawbone Range, 5 Km north-west of Buxton, 4-II-1979, *R. Pilson*, H; **New Zealand**: North Island: Ruahine-Cook Botanical District, Ruahine Range, Mount Conspicuous, 12-XII-1926, *G. Einar & G. Du Rietz*, H; Ruahine-Cook Botanical District, York Bay (near Wellington), 18-XII-1926, *G. Einar & G. Du Rietz*, H; South Auckland Botanical District, Rangitolo Island, 24-IV-1927, *G. Einar & G. Du Rietz*, H; South Island: Eastern Botanical District, Banks subdistrict, 7-I-1927, *G. Einar & G. Du Rietz*, H; Eastern Botanical District. Cass, Mount Misery, 9-I-1927, *G. Einar & G. Du Rietz*, H; Fiord Botanical Distrcit, Lower Routeburn Valley, 14-II-1927, *G. Einar & G. Du Rietz*, H; Haast-Jackson Bay Road just east of Waiatoto Lagoon, ca. 5 Km S of Hannahs Clearing, I-1999, *S. Hammer*, FH; **New Zealand**: South Island: Mt. Arthur Trail between Flora Saddle and Mt. Arthur Hut, I-1999, *S. Hammer*, FH; Mt. Arthur Trail between Flora Saddle and Mt. Arthur Hut, I-1999, *S. Hammer*, H; Mt. Arthur Trail, I-1999, *S. Hammer*, CANB 565475; Mt. Arthur Trail, I-1999, *S. Hammer*, FH; Old Whangamon Hill, blenheim side, vicinity of Nelson, XII-1998, *S. Hammer*, CANB 565479; Otago, Mt. Benger, roadside overhang, XII-2000, *S. Hammer*, H; Rolleston Track, Lewis Pass, I-1999, *S. Hammer*, FH; Tantagee Forest Road Vicinity of Nelson, I-1999, *S. Hammer*, CANB 565482; Tantagee Forest Road Vicinity of Nelson, I-1999, *S. Hammer*, FH; Westland, Hwy. 6 vicinity of Nelson, XII-1998, *S. Hammer*, FH, H; Whangamon Road, vicinity of Nelson, XII-1998, *S. Hammer*, CANB 565478, FH, H.

***Cladonia gracilis* subsp. *turbinata* (Ach.) Ahti**

Austria: VIII-1936, *Van Iterson*, L 794573; **Canada**: Northwest Territories: Mackensie Valley, Fort Simpson, 5-VI-1972, *A. H. Marsh*, H; Mackenzie District, Campbell Lake, 3-VIII-1966, *G. W. Scotter*, H; Mackenzie District, Dolomite Lake, 6-VIII-1965, *G. W. Scotter*, H; Vicinity of Glaciar (Brintnell) Lake, Britnell Lake, south shore, base of Colonel Mt., VII-1939, *L. C. Raup*, H; **China**: Heilungkiang (Heilongjiang): Yichun region, Wuying, Fungling Park, 24-XIII-1980, *L. Hämet-Ahti*, H; Jilin (Kirin): An-tu, Mt. Chang Bai, 21-IX-1981, *T. Koponen*, H; An-tu, Mt. Chang Bai, 21-IX-1981, *T. Koponen*, H; **Finland**: Pirkanmaa: Hämeenlinna, Lammi, Porraskoski, Haukkavuori, 23-VII-2009, *V. Haikonen*, H; Hausjärvi, Lavinto, Miehonvuori, 15-V-2003, *V. Haikonen*, H; Kalvola, Könnölä, Kaivolampi, 18-IV-2008, *H. Väre*, H; Kalvola, Kotkajärvi, Pastinkangas E, 19-IV-2008, *H. Väre*, H; Kalvola, Kotkajärvi, Pastinkangas, 19-IV-2008, *H. Väre*, H; Vesilahti, Kirveslami, Vuolisjärvenvuori, 22-IX-2002, *M. Kääntönen*, H; Satakunta: Isojoki, Lauhanvuori mounttain, 19-IX-1992, *T. Ahti & A. R. Burgaz*, MACB 50778; South Ostrobothnia: Isojoki, Lauhanvour i National Park, Kivijata, 19-IX-1992, *T. Ahti & A. R. Burgaz*, MACB 50776; Isojoki, W Slope of hill Lauhanvuori, Huhtakorvenkivijata, 19-IX-1992, *T. Ahti & A. R. Burgaz*, MACB 50777; Uusima: Nurmijärvi, Klaukkala Isosud, raised bog, 19-VI-1990, *T. Ahti, A. R. Burgaz, E. Fuertes & Isoviita*, MACB 50775; Artjärvi, Metsäkulma, Tähtikallio, 25-V-2008, *V. Haikonen*, H; Mäntsälä, Numminen, Riihikorvennummi, 10-V-2009, *V. Haikonen*, H; Orimattila,

Järvikylä, Vuorenmäki, 19-XI-2006, *V. Haikonen*, H; Orimattila, Kaitala, Kairesuonkangas, 20-XI-2005, *V. Haikonen*, H; Orimattila, Keituri, Kivikallio, 5-X-2002, *V. Haikonen*, H; Pukkila, Haarajoki, Venumetsä, 8-X-2008, *V. Haikonen*, H; **Japan**: Honshu: Kai, Mt. Kokushi, higashi-Yamanashi-gun, 25-VII-1972, *H. Shibuichi & K. Yoshida*, H; Toyama Pref., Nakashinkawa-gum, Tateyama-ocho, Chubu Sangaku Natural Park, Mt. Tateyama, Midagahara, 15-VIII-1970, *T. Koponen*, H; Toyama, Nakashinkawa-gum, Mt. Tateyama-cho, Chubu Sangaku Natural Park, Mt. Tateyama, 15-VIII-1970, *T. Koponen*, H; Ishikari: Hokkaido, Akan National Park, Meakan Nonaka Spa, 21-VIII-19991, *P. Alanko*, H; Hokkaido, Daisetsuzan Natural Park, 27-VIII-1972, *T. Ahti*, H; Hokkaido, Furano, Yamabe, Tokyo University Experimental forest, Mt. Dairoku, 1-IX-1972, *T. Ahti*, H; Hokkaido, Kamikawa District, Kamikawa-ocho, Mt. Daisetsu Nature Park, Kogenonsen, 10-VIII-1970, *A. Koponen & T. Koponen*, H; **Korea**: Kangwon: En route from Mt. Daechongbong to Hiungag hut, Mt. Sorak, Sokcho city, 16-VII-1996, *K. H. Moon & H. Kashiwadani*, H; **Norway**: Finmark: Vest-Finmark, Kistrand, 19 Km S of Lakselv, Revfossnes, Garesguolba, 1-VII-1960, *T. Ahti*, H; Østlandet: Akershus, Frogn hd, Söndre Hallangen, Marikova, 5-VI-1960, *T. Ahti*, H; Akershus, Frogn hd, Söndre Hallangen, Marikova, 5-VI-1960, *T. Ahti*, H; Sunnmøre: Trs. Storfjord, about 1 Km north of Helligskogen, 13-VIII-1959, *R. L. Hämet*, H; **Russia**: Leningrad: Karelia australis, Vyborg District, Berëzovye Island Reserve, Bol'shoy Berëzovyy Island, Bukhta Zakatnaya, 13-VIII-2008, *T. Ahti*, H; Perm Krai: The Perm area, city Perm, green zone of city, 17-IX-2005, *L. Gagarina*, H; Sakha Republic: Yakutia, Namskiy District (Ulus), Khomustakh, ca. 5 Km S, on high bank of Lena River valley, 11-VIII-2005, *T. Ahti & A. P. Efimova*, H; Sakhalin Oblast: Far East, Kurile Island, Kunashir Island, Lower reaches of the Kislij Rutschvej River, 26-VIII-2003, *S. Abrahamczyk*, H; Far East, Kurile Island, Kunashir Island, Saratovka River, 22-VIII-2003, *S. Abrahamczyk*, H; **Sweden**: Dalarma: Norrbárke Hagge, 22-IX-1974, *E. Wieslander*, H; Transtrand par, Hamrarbyn. På stenblock i blandskog, 9-IX-1973, *E. Wieslander*, UPS L-153344; Jämtland: Handöl, Handölsfallen, 11-VIII-1975, *T. Ahti*, H; Kölvallen, Kölvallen, 14-VI-1976, *K. Wissen & M. Wissen*, L 669296; Lappland: Jokkmokk, 7-VII-1968, *E. Wieslander*, H; Ludvika: Ludvika, Gräsberg, 8-XI-1965, *E. Wieslander*, H; Lule Lappmark: Jokkmokk par., Lilla Luleälv Parkijaure, S-sidan av sjön mitt emot Atjek., 30-VI-1966, *G. Gilenstam*, UPS L-144696; Jokkmokk, Muddus National Park, 10 Km N of Messaure, Maskokarso Canyon, 15-VIII-1959, *T. Ahti & E. Uggla*, H; Jokkmokk, Parish Messaure, N bank of Stora Luleälven ca. 8 Km WNW of Messaure Dam, 14-VIII-1959, *T. Ahti, E. Uggla & F. Skincke*, H; Småland: Hovmantorp, SE of Växjö, 19-X-1968, *M. S. Chistiansen*, H; **United States**: Alaska: Bering Land Bridge National Preserve, 17-VI-2003, *E. A. Holt*, H; Chugach National Forest, Seward Range District, one mile up Ressurection Trail Road, Seward Hiway, 7-VIII-1994, *C. Derr*, H; Twenty Mile River, Turnagain Arm, 26-VII-1993, *C. Derr*, H; Minnesota: Muskeg Lake, campsite on penninsula in the middle of the lake, 8-VIII-1996, *M. S. Cole*, H; New York??: West Hill, Preemption Rd., Chuyler Co., 18-VIII-1962, *C. F. Reed*, H; Wisconsin: Lincoln Co., Rock Falls, Grandfather Dam, along E shore of Wisconsin River along state Hwy 107, 0.7 mile south of County Hwy E., 27-IV-2002, *T. Ahti*, H; **Unknown**: *W. Nylander*, H; 1932, *Sandstede*, H.

***Cladonia gracilis* subsp. *valderiensis* Ahti**

Argentina: Gobernación del Río Negro: Lago Nahuel Huapí, 30-XI-1937, *A. Kalela*, H; Lago Nahuel Huapí, 30-XI-1937, *A. Kalela*, H; Neuquén: Nahuel Huapí National Park, Puerto Blest, laguna Los Cántaros, 30-X-1983, *L. Hämet-Ahti*, H; **Chile**: Cautín: Caburgua, II-1969, *J. Redon*, H; Llanquihué: Pargua, IX-1968, *F. J. Redon*, H; Pargua, IX-1968, *F. J. Redon*, H; Petrohúe, IX-1968, *F. J. Redon*, H; Puerto Manzano, III-1970, *F. J. Redon*, H; Puerto Varas, 9-I-1978, *H. Schindler*, H; Puerto Varas, Lago Todos los Santos, 11-I-1978, *H. Schindler*, H; Malleco: Depto. Angol, P. Nac. de Nahuelbuta, 13-III-1971, *M. Mahu*, H; P. Nac. de Nahuelbuta, 13-III-1971, *M. Mahu*, H; P. Nac. de Nahuelbuta, 28-III-1971, *M. Mahu*, H; P. Nac. de Nahuelbuta, La Vanería, 30-III-1971, *M. Mahu*, H; Osorno: Lago Rupanco, IX-1968, *J. Redon*, H; Valdivia: Choshuenco, Enco, 21-XI-1986, *G. Guzmán-Grimakli*, H.

***Cladonia gracilis* subsp. *vulnerata* Ahti**

Canada: British Columbia: Moresby Island, Base of Moresby Mt. On north side, 1-VII-1967, *I. M. Brodo, M. J. Schepanek & W. B. Schofield*, H; Oresby Island, Mount Moresby, Cirque lake at SW side of mountain, on shore of lake, 16-VI-1988, *I. M. Brodo*, H; Queen Charlote Island, Moresby Island, Tasu Sound, Fairfax Inlet, 3 Km SE of Tasu, 27-VII-1980, *T. Ahti*, H; Queen Charlotte Island, Graham Island, NE part, about 4 miles E of Masset, 4-VIII-1966, *H. Sjörs*, H, H; Queen Charlotte Island, Moresby Island, Tasu Sound, Fairfax, 3 Km SE of Tasu, 27-VII-1980, *T. Ahti*, H; Queen Charlotte Island, Moresby Island, Tasu Sound, c. 2.5 Km SW of Tasu, N slope of the summit of "Mine Mtn.", 26-VII-1980, *T. Ahti & H. Roemer*, H; South of Terrace along old Highway 25 about 1 mile north of Williams Creek bridge, 25-VII-1970, *K. E. Ohlsson*, H; South side of Burke Channelwest of Bella Coola in Crayden Bay (across from Labouchere Channel), 5-VII-1970, *K. E. Ohlsson*, H; Vancouver Island, c. 10 Km E of Port Alberni on Highway 4, "The Hump", 18-VI-1984, *T. Ahti & W. J. Noble*, H; Vancouver Island, Larry Lake above east shore of Kennedy Lake by Highway 4, 18 Km NNE of Ucluelet, 18-VI-1984, *T. Ahti & W. J. Noble*, H; Vancouver Island, Pacific Rim National Park, Long Beach Wickaninnish Beach, 17-VI-1984, *T. Ahti & W. J. Noble*, H; Vancouver Island, Sproat Lake, c. 15 Km W of Port Alberni, 16-VI-1984, *T. Ahti & W. J. Noble*, H; Vancouver Island, Sutton Pass, by Highway 4, c. 10 Km W of Sprout Lake, 18-VI-1984, *T. Ahti & W. J. Noble*, H; **Russia**: Kamchatka Krai: Kamchatka ?, 20-IV-2003?, *V. A. Balkalia*, H; **United States**: Alaska: Glacier Ranger District, lower Herring Bay, Knight Island, Prince William Sound, 26-VIII-1993, *C. Derr*, H; Glacier Ranger District, Lower Herring Bay, Knight Island, Prince Williand Sound, 26-VIII-1993, *C. Derr*, H; Glacier Ranger District, Surprise Inlet, Harriman Fiord, Prince William Sound, 23-IX-1993, *C. Derr*, H; Hogg Isalnd, Kodiak District, 4-VII-2008, *K. Dillman*, H; Usof Bay, Unalaska Island, Aleutian Island, 18-VIII-2008, *S. S. Talbot & W. B. Schofield*, H; Vicinity of Blueberry Lake Campground, mile 23, Richardson Hwy, 25-VII-1967, *T. Ahti & J. W. Thomson*, H; Oregon: Eel Creek Campground, Oregon Dunes National Recreatioons area, Coos co., 4-VII-1989, *S. Hammer*, H; Eel Creek Campground, Oregon Dunes National Recreation Area, Coos Co., 10-VII-1988, *S. Hammer*, H; Washington: Deception Pass State Park, vicinity of Anacortes, Island Co., 24-VI-1989, *S. Hammer*, H.

***Cladonia hammeri* Ahti**

Andorra: Soldeu, Port d'Envalira, 19-VIII-2006, *A. R. Burgaz*, MACB 102875; **Mexico**: Baja California: Cabode Punta Banda, 1996, *T. Nash*, H; Punta Banda Peninsula, south of Ensenada, on the northeast side of Banda Peak, along the trail about half way up the mountain, 17-XI-1990, *P. Bowler*, H; Punta Banda Peninsula, south of Ensenada, on the northeast side of Banda Peak, along the trail about half way up the mountain, 17-XI-1990, *P. Bowler*, H; **Portugal**: Algarve: Monchique, Sª de Monchique, subuda a Foia, 8-XII-2006, *A. R. Burgaz*, MACB 97323; Beira Alta: La Torre, Sª da Estrela, P. Nat. de Sª da Estrela, 3-V-2007, *A. R. Burgaz*, MACB 95120; **Spain**: Ávila: San Bartolomé de Bejar, 1-VII-2007, *A. R. Burgaz*, MACB 96093; Barcelona: Montseny, Parc Natural del Montseny, 16-VIII-2006, *A. R. Burgaz*, MACB 95732; Lugo: Queixoiro, arroyo Invernal, 21-VII-2006, *A. R. Burgaz*, MACB 95737; Madrid: Lozoya, Puerto de Navafría, 15-X-2003, *A. R. Burgaz*, MACB

96639; SomoSª, Puerto de SomoSª, arroyo del Chorro, 9-XI-2007, *A. R. Burgaz*, MACB 102876; Canary Islands: Tenerife, La Esperanza, Monte de la Esperanza, corona forestal, 16-VI-2007, *A. R. Burgaz*, MACB 102877; **Turkey**: Giresum: Degirmenagzi village, 12-II-2006, *K. Kinalioglu*, H; **United States**: California: River side County, San Mateo Canuon Wilderness, Tenaja Falls, just S and along rim of the falls along trail to Tenaja Falls, 22-VI-2000, *J. C. Lendemer*, H; River side County, San Mateo Canyon Wilderness, Tenaja Falls, just S and along rim of the falls along trail to Tenaja Falls, 22-VI-2000, *J. C. Lendemer*, F; Orange County, Peninsula Range, Santa Ana Mountains, Weir Canyon (Black Star Canyon), 6-VI-2006, *K. Knudsen 6441.4*, URC 41270; Peninsula Range, San Diego, Mission Trails Regional Park, Kwaay Paay (La Mesa), 2-V-2009, *K. Knudsen 11005*, URC 204973; Peninsula Range, Santa Ana Mountains, Weir canyon, along country trail (Black Star Canyon), 6-VI-2006, *K. Knudsen 6441.1*, URC 41269; Sonoma Co., Russian River, McMurray Ranch Winery, 12-VII-2008, H, H; Sonoma Co., Russian River, McMurray Ranch Winery, 12-VII-2008, *T. Ahti* 69200, 69198, H; Marin, Co., W of Rock Springs, on west Ridgecrest Boulevard, 11-VII-2008, *T. Ahti* 68885, H; Marin Co., Point Reyes National Seashore, Pierce Point Ranch, 13-VII-2008, *T. Ahti* 69191, H; Peninsula Range, Santa Ana Mountains, Weir Canyon, Ridgeline N of Windy Ridge Road above the Toll Road, N-facing road cut along Windy Ridge Road, 16-V-2006, *K. Knudsen*, H.

***Cladonia humilis* (With) J. R. Laundon**

Andorra: Ordino, El Serrat, Vallnord, estación de esquí Pal-Arcalís, 19-VIII-2006, *A. R. Burgaz*, MACB 97324; **Croatia**: Dubrovnik-Neretva: Zamaslina, península de Pelsejac, 31-III-2010, *A. R. Burgaz*, MACB 101103; **Denmark**: Syddanmark: Fanø, Vesterhaubad, 8-VII-2004, *E. SteenHosen*, H; Zealand: Møn, Ullshale, 8-VI-1998, *A. V. Larsen, P. Corfixen, S. N. Christensen & E. S. Hansen*, H; **Germany**: Hessen: Rheingau/Taunus-kreis, *H. Mietzsch*, F C0054571F; Rheingau-Taunus-Kreis: Hamm in wentlalen H eessen, 10-I-1998, *H. Bollan*, H; Heessen, Taumus Mts. Wisper valley, 1994, *H. M. Jahns*, H; **Iceland**: Seltátrar, Tálknfjördur, 3-VI-1999, *S. Baldursdóthis & J. B. Jónsdottir*, H; Sachsen-Anhalt, Landkreis Halle/Saale, Zwischen Marl und Brachwitz in einer Kaolingrube, 3-XI-1974, *P. Scholz*, H; **India**: Jammu and Kashir: Gulmarg at khilanmarg, 3-VII-1977, *K. Dange*, H; **Ireland**: Munster: Hiberia, Killarney, 6-II-1932, *H. Lindberg*, H; **Italy**: Calabria: Catanzaro, Serra San Bruno, Bosco di Santa Maria, 8-VIII-1986, *D. Pentillo*, H; Grosseto: Capalbio, NE of Poggio Forrane along the road Marsilliana-Capalbio, 29-V-1977, *P. Uotila*, H; **Morocco**: Near Bad Berred, 3-X-1995, *P. Uotila*, H; **Netherlands**: Friesland: Wadden sea Island Vlieland, near Vianenslid, 7-IX-2002, *A. Aptroot*, H; Wadden sea Island Vlieland, near Vianenslid, 7-IX-2002, *A. Aptroot*, H; Limburg: Molenhoek along railroad near Heumense schons, 14-IX-2003, *A. Aptroot*, H; **Poland**: Western Pomerania: Luketousc, 13-VI-1986, *W. Faltynowicz*, H; Wolin Island, Swietousc, 15-VI-1978, *W. Faltynowicz*, H; **Portugal**: Algarve: Alferce, Sª. de Monchique, alcornocal, granitos, *A. R. Burgaz*, MACB 94807; Aljezur, Praia do Monte Clérigo, 9-XII-2006, *A. R. Burgaz*, MACB 94550; María Vinagre, hacia Bahía dos Tiros, 9-XII-2006, *A. R. Burgaz*, MACB 94551; Monchique, Sª de Monchique, subida a Foia, 8-XII-2006, *A. R. Burgaz*, MACB 94548; Alto Alentejo: Bencatel, Sª de Ossa, 10-XII-2006, *A. R. Burgaz*, MACB 94553; Evoramonte, 10-I-2004, *A. R. Burgaz*, MACB 92837; Pégoes, Monte das Piçarras, 9-I-2004, *A. R. Burgaz*, MACB 93029; Subida Pico São Mamede, P. Nat. São Mamede, 10-I-2004, *A. R. Burgaz*, MACB 92813; Baixo Alentejo: Cavalheiro, cabo Sardão, 9-XII-2006, *A. R. Burgaz*, MACB 97326; Cavalheiro, cabo Sardão, 9-XII-2006, *A. R. Burgaz*, MACB 94552; Cavalheiro, cabo Sardão, 9-XII-2006, *A. R. Burgaz*, MACB 97326; Beira Alta: Vale de Amoreira, Sª. da Estrela, subida a Quinta do Fragusto, 2-V-2007, *A. R. Burgaz*, MACB 98094; Vila Soeiro do Chão, valle del río Mondego, 3-V-2007, *A. R. Burgaz*, MACB 95916; Beira Litoral: Benfeite, Sª. do Açor, Fraga de Pena, Barroco de Degrainhos, 29-III-1998, *T. Ahti & A. R. Burgaz*, MACB 95784; Côja, 1-V-1999, *N. Marcos & P. Silveira*, MACB 92897; Estremadura: Azoia, Sª de Sintra, 10-I-2004, *A. R. Burgaz*, MACB 92818; Azoia, Sª de Sintra, 10-I-2004, *A. R. Burgaz*, MACB 93027, MACB 92818, MACB 92834, MACB 93835, MACB 93028; Fernao Ferra, reserva da Mata Nacional dos Medos, 7-VIII-1999, *A. R. Burgaz*, MACB 92814; Leiria, Pegoes, San Pedro de Muel, 26-VIII-1998, *A. R. Burgaz*, MACB 97076; Sª de Sintra, Monasterio de Peninha, 10-I-2004, *A. R. Burgaz*, MACB 92836, MACB 97661; Trás os Montes: Carrazedo, Sª de Nogueira, 19-II-2005, *A. R. Burgaz & J. Marques*, MACB 94706; Carrazedo, Sª de Nogueira, 19-II-2005, *A. R. Burgaz*, MACB 92895; Nogueira, Sª de Nogueira, 20-II-2005, *A. R. Burgaz*, MACB 92980, MACB 94707; Rebordainhos, Sª de Nogueira, 19-II-2005, *A. R. Burgaz*, MACB 92881; Rebordãos, Sª da Nogueira, 6-IX-2006, *A. R. Burgaz*, MACB 93870, MACB 92885; Sao Cibrão, Sª de Nogueira, 20-II-2005, *A. R. Burgaz*, MACB 93034; Madeira Islands: Funchal, between Choupana-Camacha, Levada da Serra Faial, Quinta de Vale Paraiso, along levada, 8-II-2004, *P. Alanko*, H; Paúl da Serra, Rabacál, 10-V-1980, *R. Routsalo-Aario & L. Aario*, H; Vinhaticos, 15 Km WNW of Funchal, 10-III-1991, *A. Aprtoot*, H; Azores Islands: San Miguel, Mata Douro, 8-VII-2004, *A. F. Rodriges*, H; San Miguel, near Pico da Amendoa, 10 Km NW of Ponta Delgada, VII-1986, *A. Aptroot*, H; Terceira, Monte Brasil, 6-VII-2003, *A. F. Rodrigues*, H; Terceira, Serra de Santa Barbara-Florestal, 28-XII-2004, *A. F. Rodrigues*, H; **Russia**: Leningrad region (Isthumus karelicus): Pervomaiskoe (formerly Kivernnapa), Staraya, Krepost, 20-VIII-1993, *L. Hämet & T. Ahti*, H; Sakha Republic: Yakutia, Vekhoyansk Dist., Batagay, 0.5 Km SE of airport, by preschool, 8-VIII-2006, *T. Ahti*, H; **South Africa**: Cape province: Cape Peninsula, Karbonkelberg, 8-VI-1952, *E. Esterhuysen*, H; Karadouws Mountains near Orchard, 26-VII-1944, *E. Esterhuysen*, H; **Spain**: Álava: Leza, Sª de Cantabria, subida al Pto de Herrera, 26-VII-2006, *A. R. Burgaz*, MACB 93555; Alicante: Castalla, Sª de Castalla, Xorret del Catí, 13-V-2004, *A. R. Burgaz*, MACB 95646; Asturias: Felechosa, Foces del Pino, 5-VIII-2003, *A. R. Burgaz*, MACB 95644, MACB 93832; Pola de Laviana, Pto. de la Colladona, brezal con tojo, *A. R. Burgaz*, MACB 92824; Ávila: Candeleda, pantano del Rosarito, 5-V-2007, *A. R. Burgaz*, MACB 98093; Casavieja, Fuentelecha, 9-XI-2003, *A. R. Burgaz*, MACB 92793, MACB 98138; La Hoya, alto de la Covatilla, 1-VII-2007, *A. R. Burgaz*, MACB 97589; Monsalupe, 1-V-2007, *A. R. Burgaz*, MACB 96053; Piedrahita, 21-XI-2004, *A. R. Burgaz*, MACB 92787, MACB 92985; Piedrahita, Pto. de Peñas Negras, 21-XI-2004, *A. R. Burgaz*, MACB 92982, MACB 96762; Ramacastañas, 14-XI-2004, *A. R. Burgaz*, MACB 92788; San Bartolomé de Bejar, 1-VII-2007, *A. R. Burgaz*, MACB 956663; Barcelona: Montseny, P. Nat. del Montseny, 16-VIII-2006, *A. R. Burgaz*, MACB 95738; Burgos: Covarrubias, Sª. de Covarrubias, 6-IX-2003, *A. R. Burgaz*, MACB 97984; Río de Lunada, Montes de Valmera, subida al portillo de Lunada, 25-VII-2006, *A. R. Burgaz*, MACB 97449; Santa Cruz del valle Urbión, 30-VII-1996, *A. R. Burgaz*, MACB 92790; Urrez, Sª. de Mencilla, 12-III-2008, *A. R. Burgaz*, MACB 97289; Cáceres: Casas de Miravete, Pto. de Miravete, 9-I-2004, *A. R. Burgaz*, MACB 92833; Guadalupe, Sª. de Guadalupe, subida al pico Villuercas, 4-V-2007, *A. R. Burgaz*, MACB 96012, MACB 96058; Guadalupe, Sª. de Guadalupe, subida al pico Villuercas, 4-V-2007, *A. R. Burgaz*, MACB 98098; Hoyos, Sª de Santa Olalla, 6-IV-1996, *G. Aragón, A. Herrero & I. Martínez*, MACB 92775; La Calera, Sª. de la Palomera, 5-V-2007, *A. R. Burgaz*, MACB 96059; Navalvillar de Ibor, 4-V-2007, *A. R. Burgaz*, MACB 96056; NavatraSª, Sª. de la Palomera, 5-V-2007, *A. R. Burgaz*, MACB 96055; Perales del Puerto, Sª de Gata, Pto. de Perales, 3-V-2007, *A. R. Burgaz*, MACB 98064; Perales del Puerto, Sª de Gata, Pto. de Perales, 3-V-2007, *A. R. Burgaz*, MACB 95665, MACB 95667, MACB 95666; Robledollano, 4-V-2007, *A. R. Burgaz*, MACB 96011; Robledollano, 4-V-2007, *A. R. Burgaz*, MACB 96057; Salorino, rivera de los Molinos, 9-I-2004, *A. R. Burgaz*, MACB 92839; San Martín de Trevejo, Sª de Gata, 26-VII-1997, *A. R. Burgaz*, MACB 92870; Villamiel, Sª. de Gata, arroyo de los Legares, 2-IV-1996, *G. Aragón, A. Herrero & I. Martínez*, MACB 97325; Villar del Pedroso, Garganta del Mesta, 4-I-1995, *G. Aragón & I. Martínez*, MACB 97535; Villarreal de San Carlos, P. Nat. de

la Sª. de Monfragüe, 4-V-2007, *A. R. Burgaz*, MACB 98096, MACB 98097; Cádiz: Alcalá de los Gazules, El Picacho, P. Nat. de los Alcornocales, 21-IX-2004, *A. R. Burgaz*, MACB 98140, MACB 92805; Barbate, El Soto, 25-V-1997, *A. R. Burgaz*, MACB 92771, MACB 98158; Cortes de la Frontera, Sª. del Algibe, 4-XI-2005, *R. Pino-Bodas*, MACB 97201; Cantabria: Camaleño, Fuentedé, P. Nac. Picos de Europa, 15-XI-2003, *G. Amo, A. R. Burgaz, I. Martínez & M. G. Otálora*, MACB 89750; Entrambasaguas, Sª de Peñalabra, río Hijar, 24-VII-2006, *A. R. Burgaz*, MACB 93553; Rocamundo, 19-VIII-2008, *A. R. Burgaz*, MACB 98297; Vega de Liébana, Porcieda, 6-VI-1994, *A. R. Burgaz*, MACB 97985; Vega de Liébana, Tudes, 6-VI-1994, *A. R. Burgaz*, MACB 97941; Castellón: Artana, Sª se Espadán, 11-IV-2003, *A. R. Burgaz*, MACB 95647; Ciudad Real: Puebla de Don Rodrigo, Riofrío, 16-VI-1996, *F. J. Sarrión*, MACB 97162; Saceruela, Sª de Canalizos, 24-IX-2004, *A. R. Burgaz*, MACB 92792, MACB 98141; Solana del Pino, Sª. de Solana del Pino, 2-II-1997, *A. R. Burgaz*, MACB 96111; Villamanrique, Sª Morena, 6-II-2003, *A. R. Burgaz*, MACB 92769, 92872; Villarrubia de los Ojos, Pto. de los Santos, Sª de la Cueva, 5-XI-2004, *A. R. Burgaz*, MACB 93032; Viso del Marqués, Sª. de San Andrés, Fresnedas Altas, 6-XII-2006, *A. R. Burgaz*, MACB 98095; Córdoba: Fuente Obejuna, Valdeinfierno, 23-IV-2006, *A. R. Burgaz*, MACB 93835; Villaharta, fuente de La Lastrilla, 24-IX-2004, *A. R. Burgaz*, MACB 98142, MACB 92795; Cuenca: Campillo de Altobuey, 6-IV-2003, *A. R. Burgaz*, MACB 97986; San Clemente, 11-V-2004, *A. R. Burgaz*, MACB 92977, MACB 92979, MACB 93025; Gerona: El Masos de Pals, El Baix Empordan, 10-IX-2007, *A. R. Burgaz*, MACB 96027; Port de la Selva, L' Alt Empordan, S. Pere de Rodes, 4-IX-2007, *A. R. Burgaz*, MACB 95931, MACB 95931, MACB 96021; Sant Martí Vell, El Girones, Santuario Els Angels, 5-IX-2007, *A. R. Burgaz*, MACB 95932; Sant Sadurní d'Osormort, La Selva, 15-IX-2007, *A. R. Burgaz*, MACB 95933; Santa Pau, volcán de Sta. Mragarita, 10-XII-1995, *G. Aragón, A. Herrero & I. Martínez*, MACB 95912; Santa Pau, volcán de Sta. Margarita, 5-II-1998, *A. R. Burgaz*, MACB 97593; Granada: Alfacar, Sª de Huetor, P. Nat. de Huetor, 20-IV-2006, *A. R. Burgaz*, MACB 95668, MACB 93012, MACB 93834; Güejar-Sª, Bco. de San Juan, P. Nat. Sª Nevada, 22-IV-2006, *A. R. Burgaz*, MACB 93010; Huetor, Sª de Huetor, P. Nat. de Huetor, Fuente Fría, 20-IV-2006, *A. R. Burgaz*, MACB 95669; Lugros, Bco. de las Rozas, P. Nac. Sª Nevada, 21-IV-2006, *A. R. Burgaz*, MACB 93011; Lugros, Dehesa del Camarote, Bco. de las Rozas, P. Nac. Sª Nevada, 21-IV-2006, *A. R. Burgaz*, MACB 93074, MACB 93833; Guadalajara: Cantalojas, P. Nat. Tejera Negra, 23-VIII-2003, *A. R. Burgaz*, MACB 95860; Gascueña de Bornoba, Sª. de Alto Rey, 23-VIII-2003, *A. R. Burgaz*, MACB 97983; Hiendelaencina, 22-IV-2007, *A. R. Burgaz*, MACB 96095; Jadraque, 22-IV-2007, *A. R. Burgaz*, MACB 98031; Huelva: Castaño del Robledo, 27-IV-1993, *I. Martínez*, MACB 92794; Cortegana, I-2000, *M. P. Jones*, MACB 98082; Jaén: Albanchez de Mágina, Pto Albanchez, Sª de Mágina, 6-XII-2006, *A. R. Burgaz*, MACB 94554 La Aliseda, 26-IV-2001, *A. R. Burgaz, M. Carrasco & E. Fuertes*, MACB 92797; Montizón, cauce del río Dañador, 6-II-2003, *A. R. Burgaz*, MACB 92798; Santa Elena, Sª Morena, 23-IV-2006, *A. R. Burgaz*, MACB 93013, MACB 93030, MACB 93831; La Rioja: Anguiano-Mansilla de la Sª, valle del río Najerilla, 8-IX-2004, *A. R. Burgaz*, MACB 98036; Valdezcaray, Sª. de la Demanda, 30-X-2002, *A. R. Burgaz*, MACB 97943; Villoslada de Cameros, Sª. de Cebollera, 8-XII-2007, *A. R. Burgaz*, MACB 97268; León: Colinas del Campo de Martín Moro, 21-X-1994, *A. R. Burgaz & I. Martínez*, MACB 92799; Manzanal del Puerto, 3-IX-2002, *A. R. Burgaz*, MACB 98079; Lugo: San Martín de Suarna, 21-VII-2006, *A. R. Burgaz*, MACB 93549, MACB 93550, MACB 93554; Madrid: Buitrago de Lozoya, 26-XI-1989, *A. R. Burgaz*, MACB 37181; Bustarviejo, Pº. de Canencia, arroyo del Sestil del Mahillo, 30-I-1994, *G. Aragón & I. Martínez*, MACB 96024; Colmenar de Oreja, 10-X-1999, *A. R. Burgaz*, MACB 96714; El Berrueco, 9-XI-2007, *A. R. Burgaz*, MACB 96010; El Pardo, monte de El Pardo, 19-II-2008, *A. R. Burgaz*, MACB 97662, MACB 97662; Hoyo de Manzanares, 1-XI-1989, *A. R. Burgaz*, MACB 37180; La Acebeda, Sª de SomoSª, camino del Pto de la Acebeda, 14-I-2007, *A. R. Burgaz*, MACB 95913, MACB 95913; Monte de El Pardo, 9-IV-2004, *A. R. Bugaz*, MACB 92801; Montejo de la Sª, 8-III-2002, *P. Aguilar, G. Amo & A. R. Burgaz*, MACB 92874; SomoSª, Pto. de SomoSª, arroyo de la Peña del Chorro, 9-XI-2007, *A. R. Burgaz*, MACB 98034; Torrelodones, 22-V-1995, *A. R. Burgaz*, MACB 92800; Málaga: Parauta, Sª de las Nieves, 20-III-1995, *I. Martínez*, MACB 92802; Pujerra, Sª. Bermeja, 10-V-2005, *A. R. Burgaz*, MACB 96764; Navarra: Lizarraga, bajada Pto. de Lizarraga, Sª. de Andía, 25-VII-1994, *A. R. Burgaz*, MACB 97995; Puerto de Velate, valle de Ultzama, 9-IX-1991, *T. Ahti & A. R. Burgaz*, MACB 98035; Orense: subida Cabeza de Manzaneda, Sª de Queixa, 24-VIII-2002, *A. R. Burgaz*, MACB 92774; Palencia: Resoba, Resrva Nac. Fuentes Carrionas, 24-VII-2006, *A. R. Burgaz*, MACB 9355, MACB 93552; Pontevedra: Isla de Ons, 27-VIII-2002, *A. R. Burgaz*, MACB 92811, MACB 98081; La Guardia, Monte de Sta. Tecla, 29-VIII-2002, *A. R. Burgaz*, MACB 92880; Salamanca: Beleña, 5-XI-2005, *A. R. Burgaz*, MACB 92826; Carpio de Azaba, 1-V-2007, *A. R. Burgaz*, MACB 96671, MACB 96054, MACB 98083; El Cabaco, Sª de la Peña de Francia, 6-XI-2005, *A. R. Burgaz*, MACB 92831, MACB 92974; El Casarito, Sª. de la Peña de Francia, 6-XI-2005, *A. R. Burgaz*, MACB 97944; Frades de la Sª, Sª de Frades, 5-XI-2005, *A. R. Burgaz*, MACB 92830; Fuenterroble de Salvatierra, 5-XI-2005, *A. R. Burgaz*, MACB 92829, MACB 92827, MACB 92973; La Alberca, Sª. de las Mestas, Pº. del Portillo, 6-XI-2005, *A. R. Burgaz*, MACB 95785; Sancti Spiritus, 1-V-2007, *A. R. Burgaz*, MACB 95914, MACB 98099; Valdemierque, 5-XI-2005, *A. R. Burgaz*, MACB 92812, MACB 92825, MACB 98039; Segovia: Aldehuela de Pedraza, subida al Pto. de Navafría, 26-I-2008, *A. R. Burgaz*, MACB 97945; El Espinar, valle del río Moros, área recreativa La Panera, 26-I-2008, *A. R. Burgaz*, MACB 96801, MACB 96802; La Granja de San Ildefonso, 11-XI-2005, *R. Pino-Bodas*, MACB 97663; La Granja, Valsaín, río Eresma, fuente de los Dos Caños, 26-I-2008, *A. R. Burgaz*, MACB 97059; Navafría, subida a la cascada del Chorro, 26-I-2008, *A. R. Burgaz*, MACB 97636; Segovia, Revenga, cordel de Peñas Zamarriegas, 26-I-2008, *A. R. Burgaz*, MACB 96803; Sevilla: Alanis, Mirador Loma del Aire, P. Nat. de Sª Norte, 23-IV-2006, *A. R. Burgaz*, MACB 93836; Cazalla de la Sª, Finca UPA, P. Nat. Sª Norte, 23-IV-2006, *A. R. Burgaz*, MACB 93016; La Puebla del Río, dehesa de Abajo, 7-XII-2006, *A. R. Burgaz*, MACB 94555; Soria: Lubia, Altos de Lubia, 7-IX-2003, *A. R. Burgaz*, MACB 98040; Matalebreras, 13-IX-2003, *A. R. Burgaz*, MACB 97982, MACB 98065; Pto. de Santa Inés, 7-IX-2003, *A. R. Burgaz*, MACB 92858; Tarragona: La Sénia, El Retaule, 25-VI-1992, *A. R. Burgaz*, MACB 95915; Teruel: Orihuela del Tremedal, Sª. de Albarracín, arroyo Gargantavellanos, 19-V-1996, *G. Aragón, A. Herrero & I. Martínez*, MACB 97269; Toledo: Belvis de la Jara, 30-IX-2005, *R. Pino-Bodas*, MACB 97594; Consuegra, Sª de Valdehierro, 5-XI-2004, *A. R. Burgaz*, MACB 92806; La Iglesuela, 23-III-2003, *A. R. Burgaz*, MACB 92777; La Iglesuela, 23-III-2003, *A. R. Burgaz*, MACB 98168; Los Navalucillos, Montes de Toledo, arroyo del Chorro, 11-II-1995, *G. Aragón, A. Herrero & I. Martínez*, MACB 97270; Robledo del Mazo, 15-II-2006, *R. Pino-Bodas*, MACB 92968; Urda, Sª de Morrones, finca El Convento, 5-XI-2004, *A. R. Burgaz*, MACB 92968; Urda, Sª de Morrones, subida antena T.V., 5-XI-2004, *A. R. Burgaz*, MACB 92807, MACB 92816, MACB 92807; Urda, Sª Morrones, finca El Convento, 5-XI-2004, *A. R. Burgaz*, MACB 92776; Valencia: Benagéber, Sª. del Negrete, valle del Guadalaviar, 28-II-2009, *A. R. Burgaz*, MACB 99821; Zaragoza: Tarazona, Sª del Moncayo, 20-IV-1993, *A. R. Burgaz*, MACB 92810; Vera de Moncayo, Sª. de Moncayo, 13-IX-2003, *A. R. Burgaz*, MACB 97981; Canary Islands: La Gomera, P. Nac. de Garajonay, Degollada Blanca, entre Igualen y las Tajoras, XII-2002, *Hernandez-Padrón & P. L. Pérez de Paz*, MACB 93492; Bareares Islands: Mallorca, Pollença, carretera de cabo Formentor, 11-I-2006, *A. R. Burgaz*, MACB 92878; Pollença, El Mal Pas, 11-I-2006, *A. R. Burgaz*, MACB 92877; Sa Pobla, 11-I-2006, *A. R. Burgaz*, MACB 92803, 92804; Sa Pobla, 11-I-2006, *A. R. Burgaz*, MACB 92803; Santa Margarita, Son Serra de Marina, 10-I-2006, *A. R. Burgaz*, MACB 92875, 92876; Menorca, Ferreríes, subida a la ermita, 28-XI-2007, *A. R. Burgaz*, MACB 96765; Ibiza, Sant Joseph de sa Talaia, monte de sa Talaia, 12-IX-2008, *A. R. Burgaz*, MACB 98234, MACB 98296; **Sweden**: Göteborg: Götebarg, Gem. Vestra Frölunda, Auf den Trümmern eines Kl. Haufes, fuhr trooken und winding belegen,

10-IV-1922, *C. Stenholm*, H; **Turkey**: Central Anatolia: Akyarm, 120 Km NW of Ankara on the road to Istanbul, 29-VIII-1972, *P. Uotila*, H; Sakaray: Sapanca lake, 10-X-1994, *A. Ciçek*, H; Tekirdag: Sarköy, Yeniköy, by the main road, 10 Km NW of Sarköy, c. 5 Km of Yeniköy, 15-V-1990, *P. Uotila*, H; Trabzon: Akcaabat town, Kemaliye village, VI-2005, *K. Yazici*, H; **United Kingdom**: awden Hill, 7-IV-1974, *M. Seaward & B. J. Coppins*, H; England: Breidden Hill, dolorite sand by road, 21-X-1961, *T. D. V. Swinscow*, H; West Cornwall, V. C. 1. Isles of Scilly, St. Mary´s Peninnis Head, 4-V-1979, *P. W. James*, H; Scothland: Berwickshire (V. C. 81) Cockburas path, 1-VIII-1964, *T. Ahti*, H.

***Cladonia macroceras* (Delise) Hav.**

Andorra: Soldeu, Port d'Envalira, 19-VIII-2006, *A. R. Burgaz*, MACB 94200; **Austria**: Einen Rasen bildend auf bemoostem Kalkboden am Grunde einer jungen Zierbe am Abbange ober der Alpe Vineghia bei Panaveggio in Südtirol, 2-VIII-1886, *Arnold*, H; Kärnten Pad 338 r. Schwartzstein, N of Emberger, Weißbriach, 24-VII-1994, *L. Spier*, L 753010; Österreich: Niedere Tauern, Steiermark, Wölzer, N-Abhänge des HochgröBen, 13-VI-1989, *J. Hafellner*, H; Steiermark, Styria, Niedere Tauem, Untertal, near Schladming, 8-VIII-1985, *H. Köckinger*, H; Tirol, Silvretta-Gruppe, Jamtal, oberhalb der Jamtalhütte, grober Blockschutt unterhalb des "Steinmannli", 13-VIII-1983, *T. Feuerer*, H; Tirol, Stubaier Alpen, Nederbachtal, am Weg von der StraBe zur Balbach-Sennhütte, 5-VIII-1986, *T. Feuerer*, H; Salzburg: Salzburg, Kitzbühler Schieferalpen W of Mt. Schmittenhöhe above Zell am See, N and above Pinzgauer Hütte, 9-IX-1973, *O. Vitikainen*, H; Styria: Steiermark, Seetaler Alpen, 10 Km WNW of Obdach, road from Schmelz to Winterleitenhütte, 28-V-2003, *W. Obermayer*, H; Tyrol: Stubaier Alpen, Gschnitztal, Trins, on the road to Padasterjoch, 12-IX-1973, *O. Vitikainen*, H; Im Kiefelfchiefergeröll des Jefchkenkegels auf Humuserden, 14-VII-1918, *J. Anders*, H; **China**: Xinjiang: Tianshan Mountains, Shawan, 2-VII-2002, *A. Abbas*, FH; **Finland**: Mainhomm?, 1903, *F. W. Khingstedt*, H; Nauelann, 1903, *F. W. Khingstedt*, H; Patsjohistrono, vajrousen, 12-II-1909, *A. Renvall*, H; Peninsula Piseatorum, Rattolahti, Mys-Kekurkij, 29-VI-1909, *F. W. Klingstedt*, H; Finland Proper: Korpo, islet Närmast Fjälskär NE of Brunskär, 7-VII-1988, *T. Ahti*, H; Kuusamo: Juuma, Jäkälävouma gorge, W end, 12-VI-1980, *T. Ahti*, H; Lapland: Enare, 1910, *A. Renvall*, H; Enontekiö Lapland, Enontekiö, Mt. Saana, NE-side, 26-VII-2005, *H. Väre*, H; Enontekiö, Kilpisjärvi, N-Jehekats, 3-VIII-1949, *A. J. Huuskonen*, H; Enontekiö, Kilpisjärvi, Saanan SW-rinteellä kivikossa, 31-VII-1955, *T. Ahti*, H; Enontekiö, Kilpisjärvi, W-Saana, 17-VII-1964, *M. Haapasaari*, H; Enontekiö, Kilpisjärvi, W-Saana, paljakan pahta, 17-VII-1964, *M. Haapasaari*, H; Enontekiön Lappi, S-Tschaima, 9-VIII-1959, *A. J. Huuskonen*, H; Inari, Sevettijärvivägen, Vid Ukonselkäs utlopp, 22-VII-1974, *G. Korit*, H; Kilpisjärvi, Saana, SW-Hang, 22-VI-1955, *A. Henssen*, H; Muonio, Katkesnianto, Pahtarime, 1867, *J. P.Norrlin*, H; Par. Munioniska, Pahtarine prope Katkesnanlo, 1867, *J. P.Norrlin*, H; Par. Muonioniska, 1867, *J. P. Norrlin*, H; Ponojensis ad promontorium Orlow, 25-V-1989, *A. O. Kihlman*, H; **France**: Aquitaine: Pyrénées Atlantiques Ste. Engrâce, 4-VIII-1992, *L. Spier*, L 753011; Hautes-Alpes: Champoléon, Puy de la Chaumette, 10-IX-1978, *Y. Rodon*, H; Le Valgademar, Combefroide, 26-IX-1977, *Y. Rodon*, H; **Germany**: 1932, *H. Sandsdede*, H; **Mongolia**: Borsag Valley off of Lake Hovsgul, north of Hatgal, VI-2005, *J. Belnap*, H; Ulan Bator City, NW Slope of Mt. Bogd Uul, Dzaysan Canyon, ca. 2-4 Km SW of Dzaysan hill Monument, 10-VII-1972, *T. Norlindh & T. Ahti*, S F84189; **Poland**: Cracow Artic, Svalbard, Spitsbergen, Hornsund, Ralstranda, sea terrace between Jakobsenpyten and Lakpynten, VII-2002, *P. Osyczka*, H; Lesser Poland: Nowy Sacz prov., High Tatra, Tatra National Park, Mt. Kasprowy Wierch, c. 1 Km W of peak, 25-X-1986, *T. Ahti & M. Olech*, H; Polish Tatra Mountains (distr. Nowy Targ.) Stawy Gasienicowe Valley, Moraine of Czarny Staw lake, 26-VII-1971, *J. Nowak*, H; **Russia**: Altai: Western part, at western part of Tigirek range, 23-VII-1996, *E. A. Davydov*, H; Kamchatka Krai: Kamchatka, Southern slope of Tolbachik Volcano, 7-VIII-2006, *D. Himelbrant & I. Stepanchikova*, H; N of Central Siberia, Severnaya Zemlya Archipelago, W coast of Oktyabr'skaya Revolutsiya Is., watershed at Peschanaya River basin, 17-VII-1985, *M. Gavilo*, H; Krasnoyark: North of Central Siberia, Severmaya Zemlya Archipelago, N Part of Bol'shevik IS., W coast of Akhmatov Bay, 7 Km SW of the Bazovaya River mouth, 18-VII-996, *M. Zhurbenko*, H; North of Central Siberia, Severnaya Zemlya Archipelago, southern coast of Bol'shevik Is. Opposite Cape Chelyuskin at Taimyr cosat, 28-VIII-2000, *N. Matveeva*, H; North of Central Siberia, severnaya Zemlyan Archipelago, Southern coast of ol'shevik Is. Opposite Cape Chelyuskin at Taimyr coast, 14-VIII-1997, *N. Matveeva*, H; North of Central Siberia, Taimyr Peninsula, near Belyi Yar at the north-western coast of Pyasino Lake in a vicinities of Nyapan hills, 26-VII-1999, *L. Zanokha*, H; **Spain**: Barcelona: Montseny, P. Nat. del Montseny, Turó de l'Home, 16-VIII-2006, *A. R. Burgaz*, MACB 94197; Gerona: Setcases, estación de esquí Valter 2000, 17-VIII-2006, *A. R. Burgaz*, MACB 94199, MACB 94198, MACB 102864; Huesca: Sallent de Gállego, Campo de HTraya, 8-IX-1994, *A. R. Burgaz*, MACB 96781; Madrid: Rascafría, Sª de Guadarrama, P. Nat. de Peñalara, subida a la cumbre de Peñalara, 29-IV-2001, *I. Martínez*, MACB 96687; Rascafría, Sª de Guadarrama, P. Nat. de Peñalara, cumbre de Peñalara, 30-IV-2006, *A. R. Burgaz*, MACB 102860; Rascafría, Sª de Guadarrama, P. Nat. de Peñalara, cumbre Hermana Mayor, 30-IV-2006, *A. R. Burgaz*, MACB 102861, MACB 102862, MACB 102863; Zaragoza: Circo de San Miguel, Sª del Moncayo, 13-IX-2003, *A. R. Burgaz*, MACB 102896, MACB 102897, MACB 102898; **Sweden**: Gotland: Eksta par., Stora Karlsö, Röjsu haid, 24-VI-1943, *G. Degelius*, UPS L-74078; Öland: Gardby, 100 m N of crossing of road to öv. Alebäck, 2-VIII-1983, *O. Vitikainen*, MACB 64964; Sverige: Pite lappmark, Arjeplog sn, NV om Stiehpaltjahkka toppen, SSV om Sädvvájávrres NV-ände, Stuorsavvun, strax O om stig, 23-VIII-2008, *G. Odelvik*, S F61966; Torne Lappmark: Jukkasjärvi par., Pesisvare, 14-VII-1921, *A. H. Magnusson*, UPS L-144657; **Switzerland**: Bern: Ct. Bern., Simmental, St. Stephan, 3.2 Km W of St. Stephan Sta. Rinderberg, Gandlouenegrat, 25-V-1969, *T. Ahti*, H; Obwald: Canton d'Obwald, Engelberg, Trübsee, 3-IX-1972, *F. Page*, H; Wallis: Riederalp, Aletschwald, 9-IX-1982, *S. Hyvönen*, H; Riederalp, open slope NE of Hoflüe, 2-IX-1982, *S. Hyvönen*, H; Riederalp, Riederfurka, 23-VIII-1982, *S. Hyvönen*, H; Saas Fee, Plattjen, 31-VIII-1970, *F. Page*, H; Salvan, Van d'en Haut, 26-VIII-1973, *F. Page*, H.

***Cladonia macroptera* Rässämen**

China: Shaanxi: Mt.Taibai Shan, Mingxing Si, 2-VI-1963, *J. C. Wei*, H; **China**: Sichuan: Aba, Jiuzhaigou county, ca. 20 Km of the pass to Songpan county, 10-X-1999, *K. H. Moon*, H; Aba, Liuzhaigou Co., ca 20 Km of Chuanxi Temple, 8-X-1999, *K. H. Moon*, H; South-Eastern Kansu: Min-Shan Range, Jan-go, bergsmassiv a Min-Shans sydsida, övre Tebbu, 2-VIII-1930, *D. Hummel*, H; Yunnan: Lijiang Prefecture, White river, 6-VIII-1985, *W. Li-song*, H; Lijiang Prefecture, White river, 6-VIII-1985, *W.Li-song*, H; **India**: Himachal Pradesh: Kullu Dist., Great Himalayan Park, Soupdhar, 7-IX-1999, *D. K. Upreti*, H; Sikkim: Labdang forest, Mei Men Chu Lake area, 18-IX-1998, *G.P. Sinha*, H; North Sikkim Dist., The La to Jakthang way, 14-VII-2000, *G. P. Sinha*, H; West Sikkim Distr., on away between Dzongri-Thagsing, 15-X-1995, *G. P. Sinha*, H; **Japan**: Honshu: Chubu, Yatsugatake, 8-VII-1930, *A. Yaruda*, H; **Nepal**: Eastern region: Mechi, Montée de Mai Pokhari (clairère) sur lacrete de Chintapu et au col, puis suite de la crete de Chintapu et campement sous la crete, 29-IX-1971, *P. Ozenda*, H.

***Cladonia magyarica* Vainio**

Hungary: County Pest, Vácrátót, "Kis-Tece", MACB 98243; **Ukraine**: Donests'k Oblast: Shakhtarsky district, steppe slops near Petrivs'ke Village and Sevostianovka River, regional Landscape Park "Dontsk upland", 17-IV-2006, *O. Nadeina*, H.

***Cladonia maxima* (Ahsa.) Ahti**

Canada: Newfoundland: Island of Newfoundland, Avalon Peninsula, Butterpot Provincia Park, east side of Big Otter Pond, 9-IX-2007, *J. C. Lendemer*, H; **Russia**: Karelia Republic: Karelia pomorica occidentalis Kem' District, north of Kem' town, Island Zelenaya, 4-VIII-2006, *P. Utiola*, H.

***Cladonia multiformis* Merrill**

Canada: Alberta: Edmonton, raving north of Westbrook Drive, 19-X-1980, *G. W. Scotter*, H; British Columbia: Gukf Island, Mayne Island, 4 Km ESE of Willage Bay, Tinker road, 16-VII-1980, *T. Ahti*, H; Newfoundland: Confluence of Anderson and Careath Rivers, East Bank, 7-VII-1965, *G. W. Scotter*, H; Terra Nova National Park, 3 Km S of Trout Pond (Charlottetown turnoff), 25-VI-1980, *B. Hoisington & W. Maass*, H; Nova Scotia: Colcheter Co., Portapique, Wilderness Area, N of Montrose, E side of Portapique River, 17-V-2004, *T. Ahti*, H; Ontario: Attawapiskat, 24-VI-1958, *T. Ahti*, H; Kenogamisis Lake, 3-VII-1958, *T. Ahti*, H; Quebec: Riviere Rimouski, Rimouski, 14-V-1940, *E. Lepage*, H; Ste-Blandine, Co. Rimouski, 17-VI-1941, *E. Lepage*, H.

***Cladonia nashii* Ahti**

Mexico: Baja California: 2Km S de Colonet, 3-I-1989, *L. Guzmán-Dávalos*, H; San Quintín peninsula, flat below Usnea, 25-II-1999, *P. Bowler & B. Bretz*, H; **United States**: California: Santa Barbara County, Santa Rosa Island, twin Faults area 0.5 km w Lobos Canyon, 4-I-1994, *J. Marsh*, H; Santa Rosa Island, twin Fault, west of Lobos Canyon, along smith Highway, 18-X-2006, *K. Knudsen*, H; Santa Rosa Island, twin faults,west of Lobos Canyon along smith Highway, 18-X-2006, *K. Knudsen*, H; Transverse Range, Santa Cruz Island, along Torrey Pines Road, 15-X-2006, *K. Knudsen*, H; Marin Co., W of Rock Springs on West Ridgecrest Boulevard, 11-VII-2008, *T. Ahti 68967 & L. St. Clair*, H; Sonoma Co., Russian River, McMurray Ranch Winery, 12-VII-2008, *T. Ahti* 69200a, H.

***Cladonia pocillum* (Ach.) Grognot**

France: Corsica: Monte Cinto, *E. Granda*, MACB 102880; **United States**: Connecticut: Litchfield Co. 0.5 mile N CT Route, 21-IX-2003, *J. C. Lendemer & A. L. A. Foray*, UPS L-160151; Minnesota: Wabasha County, 4 miles E of Zumbro falls, 1997, *A. Tehler 7806*, S L3680.

***Cladonia pulvinata* (Sandst.) Van Herk & Aptroot**

Portugal: Beira Alta: Penhas Douradas, Sª da Estrela, P. Nat. De Sª da Estrela, 2-V-2007, A. R. Burgaz, MACB 98106, MACB 94935; Minho: Sapiãos, 12-VIII-1994, *A. R. Burgaz*, MACB 66843; Trás Os Montes: Montezinho, Sª de Montezhinho, 8-IX-2006, *R. Pino-Bodas*, MACB 94339; Montezinho, Sª de Montezhinho, 8-IX-2006, *A. R. Burgaz*, MACB 95597; **Spain:** Asturias: Villanueva, Puerto de San Lorenzo, 08-VIII-2003, *A. R. Burgaz*, MACB 91635; León: Tejedo de Ancares, 11-VII-1984, *Burgaz*, MACB 45677; Brazuelo, Montes de León, 20-VII-2006, *A. R. Burgaz*, MACB 98161; Orense: Montes do Invernadeiro, Villarino de Conso, 19-VI-1995, *A. R. Burgaz*, MACB 61612, MACB 90980; Segovia: Coca, Finca El Sequero, 20-VII-2006, *A. R. Burgaz*, MACB 95598; Riofrío de Riaza, Puerto de la Quesera, 19-VI-1991, *A. R. Burgaz*, H, MACB 45678.

***Cladonia pulvinella* S. Hammer**

Bosnia Herzegovina: Herzegovina-Neretva: Caplijna, Hutovo Blato, 29-III-2010, *A. R. Burgaz*, MACB 10115; **Greece**: Chalkidiki: Sithoniá, Sikéa, 22-VI-1987, *A. Zaharopoulou*, H; **India**: Himalaya, Singalilla, Sandakphu, Tonghu, 11-V-1959, *J. L. Van Soest 28882*, H; **Italy**: Calabria: Cosenza, Valle Fiume Jassa, 8-II-1985, *D. Puntillo*, H; Sardinia: Prov. Nuoro, Catena del Marghige, Nuraghe Ortaghis near Punta Palai, 18-VII-1987, *T. Ahti*, H; **Mexico**: Camino a Josephine Saddle, Madera Canyon, Santa Rita Mountains, Santa Cruz County, Arizona, 31-XII-1988, *I. Álvarez*, H; Baja California: 2 Km de Colonet, 3-I-1989, *L. Guzmán-Dávalos*, H; Vertiente norte del cerro de Santa Catalina, cerca de Santa Marta, Aztahuacán, 8-VII-1973, *J. Rzedowski*, H; Jalisco: 47 Km desde Bolaños, brecha Bolaños-Tenzompa, municipio de Mezquitic, Jalisco, 13-IV-1987, *L. Guzmán-Dávalos*, H; **Portugal**: Estremadura: Sª de Sintra, Monasterio de Peninha, 10-I-2004, *A. R. Burgaz*, MACB 92820; **Spain**: Almería: Rodalquilar, Sª de Cabo de Gata, 9-X-2002, *A. R. Burgaz*, MACB 97949; Menorca: Es Mercadal, subida al castillo de Sta. Agueda, 1-XII-2007, *A. R. Burgaz*, MACB 98059; Murcia: Alhama de Murcia, P. Nat. Sª Espuña, 11-V-2004, *A. R. Burgaz*, MACB 92808; Sevilla: Alanis, P. Nat. de Sª Norte, Mirador Loma del Aire, 23-IV-2006, *A. R. Burgaz*, MACB 93015; Canary Island: La Palma, Cumbre Vieja, refugio forestal, 15-II-1978, *R. Rajalin*, H; La Palma, La Cumbrecita, mirador de los Roques, 2004, *I. Perez-Vargas*, MACB 95455; **United States**: California: Monterey Cypress, Skyline Blvd., Pacifica, San Mateo Co., 29-XI-1966, *H. D. Thiers*, H; Terrestrial, Ridge Trail, PT. Reyes National Seashore ca. 1KM from ocean, Marin Co., 7-IX-1987, *S. Hammer*, H; Oregón: on compressed duff at roadside, 4.6 mi E. Of Imnaha, Wallowa co., 29-VI-1991, *S. Hammer*, H; **Venezuela**: Merida: La Sábana, area del Pico de Horma, al sureste de Mesa Quintero, 13-IV-1980, *L. Figueras & H. Rodríguez*, H.

***Cladonia pyxidata* (L.) Hoffm.**

Greenland: Qeqertaq, 2-VIII-2003, *E. S. Hansen*, H; **Ukraine:** Luhans`k Oblast: Pereval`s`k District, steppe slops between Mikhajlivka and Troits`ke Villages, near water storage basin "Isakiivake", 9-IV-2007, *O. Nadeina*, H.

***Cladonia rangiformis* Hoffm.**

Cape Verde Islands: Santo Antao: Ribe. Do Paúl, 22-VIII-1988, B. Mies, H; **Denmark**: Bornholm: Akirkeby, Klintebakken, 5-VI-2004, *E. S. Hansen*, H; Bornholm: Blykobbe Plantage, Skovly, 13-VII-2001, *E. S. Hansen*, H; Bornholm: Sandkas, 11-

VII-2002, *E. S. Hansen*, H; Fanø: Vesterhavsbad, 8-VI-2004, *E. S. Hansen*, H; Jylland: Kandestederne, 26-VI-2000, *E. S. Hansen*, H; Zealand: Asserbo Plantage, E of Staengehus, 13-XII-1994, *S. N. Christensen & E. S. Hansen*, H; **Finland**: Uusimaa: Helsinki, Vuosaari, Kalkkissaari, 20-IX-2005, *J. Pykälä*, H; **Germany**: Auf begrastem Heideboden b. Weden, Hann, X-1921, *H. Sandstede*, H; Oldenburg: In turfosis "Kehnmoor" prope Zwischenahn, *H. Sandstede*, H; **Greece**: Crete: Imbros, upper part of Imbros gorge, 29-IV-2002, *H. Väre*, H; Crete: Topolia, Katsamatodes, N of Agios Athanasios, 3-V-2002, *P. Alanko*, H; Cycladas: Santorini (Thira), Episkopi Ghonias, Profitis Ilias, 11-X-2008, *H. Väre*, H; Santorini (Thira), Perissa, Mt. Mesa Vouna, 3-X-2008, *H. Väre*, H; **Iran**: Azarbayejan: Tabriz, Jolfa toward Khoda afarin Missa village, 10-V-2003, *M. Sohrabi*, H; Azarbayejan: Tabriz, Jolfa toward Khoda afarin Missan village, 10-V-2003, *M. Sohrabi*, H; Mazandaran: Amol, toward Tehran, Haraz way, Kelerd village, 3-I-2004, M. Sohrabi, H; Mazandaran: Nour the road of Nour toward Amol, Lavij villagearound the road, 2-IV-2002, *M. Sohrabi*, H; Mazandaran: Nour, Kojdur and Kodir villages, 3-IV-2002, *M. Sohrabi*, H; **Morocco**: Chefchaouen: Chaouen, entre Bad Taza y Bad Barred, cerca de Chefarat, 3-X-1995, *P. Uotila*, H; **Netherland**: Zoid-Hollond: Stelldom, Hamipulietda, Schulhuck, 4-IX-2002, *H. Van der Goes et al.*, H; **Portugal**: Azores Islands: Sao Miguel, hauptstrasse 2 Km NW Setes Cidades, 29-VII-2001, *F. Berger*, H; Madeira Island: Paul da Serra W, along Ribeiro Janela, NW od Rabacal, 7-X-2005, *H. Väre*, H; Paul da Serra W, along Ribeiro Janela, NW od Rabacal, 7-X-2005, *H. Väre*, H; Ribeiro Frio, along path toBalcoes, 7-II-2001, P.Alanko, H; **Spain**: Baleares Islands: Menorca, Ferreires, subida a la ermita, 28-XI-2007, *A. R. Burgaz*, MACB 96193; Madrid: Carretera a La Cabrera, Valgallegos, 22-I-2009, *P. Marín & A. Vendre*, MACB 103065; Toledo: Consuegra, Sª de Valdehierro, 5-XI-2004, *A. R. Burgaz*, MACB 102951; Zaragoza: Vera de Moncayo, Sª del Moncayo, 13-IX-2003, *A. R. Burgaz*, MACB 102949; Canary Islands: Gran Canaria, Caldera de Bandama, 12-IV-2006, *H. Väre*, H; Gran Canaria, Caldera de Bandama, 12-IV-2006, *H. Väre*, H; Gran Canaria, cerca de Moya, parte superior del Baranco de la Virgen, 18-IV-2006, *H. Väre*, H; La Palma, Fuentecaliente, Fuente de los Roquedos-El Cabrito, 8-X-1999, *N. Marcos*, MACB 90888; Tenerife, La Caldera, 11-I-2008, *H. J. Reimer*, FR 77067; Baleares Islands: Mallorca, Fornalux, Sa Comuna, Sª de Tramuntana, calizas, *A. R. Burgaz*, MACB 102950; **Sweden**: Öland: Böda, stony sea shore at Toknäs udde c. 0.5 Km S of Byxelkrok, 30-V-2000, *R. Skytén*, H; **Turkey**: Afyon: 37 Km from Afyon towards Kütahya along the main road, Anikaya, Cumali Meukii, 21-V-1991, *P. Uotila*, H; Giresum: Degirmenagzi village, 12-II-2006, K. Kinalioglu, H; Zonguldak: Beycuma county, Cayköy district and surroundings, 20-VIII-2005, *K. Yazici*, H; Zonguldak: Beycuma county, Cayköy district and surroundings, 20-VIII-2005, *K. Yazici*, H.

***Cladonia rei* Schaerer**

Austria: Lower Austria: Niederösterreich, Waldviertel, Stift Altenburg, 14-IX-1994, *R. Türk*, H; Niederösterrich, Eisentadt, Hof am Leithaberge, Leithaberg, 9-IX-1994, *B. Marbach*, H; Niederösterrich, Eisentadt, Hof am Leithaberge, Leithaberg, Reisalpe SE von Lilienfeld, Weg von Brennalm zum Schutzhaus, 27-X-1995, *R. Türk*, H; Salzburg: Pongau, Bischofshofen, Buchberg, 30-X-2006, *R. Türk*, H; Tirol: Osttirol, Abfaltersbach Margarethebrück, zwischen Moosen, 19-V-1982, *A. Kofler*, H ; **Bulgaria**: Burgas: Ropotamo, 20-VIII-1973, *B. Matousek*, BRA-CR 10012; **Canada**: Newfoundland: Island of Newfoundland, Avalon Peninsula, Burry Heights Center, 7-IX-2007, *J.C. Lende,er*, BG L86394; Nova Scotia: Colchester Co., Portapique, Wilderness Area, N of Montrose, E side of Portapique River, on logging road near SE corner, 17-V-2004, *T. Ahti*, H; Ontario: West edge marathon, Shore of Lake Superior and back on rock ridges with black spruce and satteres quaking aspen, 5-VIII-2002, *C. M. Wetmore No. 88088*, S L58841; **Czech Republic**: Karlovy Vary: Slavkvský les upland, Sokolov, hormí Slavkov, quarry "Jáma Hubert" c. 1.5 Km S of town, 18-IV-2009, *J. Vondrák* 7024; Sokolvská pánev basin, Frantiskovy Lázne, protected area "Soos", 19-IV-2009, *J. Vondrák* 7026; South Bohemian: Ceskobudejovická, pánev basin, Ceské Budejovice, Nové Hodejovice, damm of settling pit above village, 2-IV-2009, *J. Vondrák* 6982; Ceský Krumlov, Nové Dobrkovice, protected area "Vysenské Kopce", 26-X-2004, *J. Vondrák, Soun, Bartos* 2368; Javorníky, in monte Tábor, 7-VIII-1981, *J. Surhanák*, BRA-CR 10026; Strední Povltaví, Týn nad Vltavou, Temelín, at railway between Temelín and nuclear power-plant "Temelin", 4-IV-2009, *J. Vondrák 7009*; Trebonká pánev basin,Veselí n/Luz, Vlkovská pískovna, 12-X-2000, *J. Vondrák*; Ceskomorav, vrchovina highlands, Velké Menirici, 2-IV-2000, *J. Vondrák*; **Germany**: Angsburg, *M. Britzelmayr*, H; **Hungary**: Csévharaszt: Praematricum, 8-V-1968, *K. Verseghy*, BRA-CR 10054; **Japan**: Akita: Honshu, Prov. Ugo, Horikawa, Iijima, Akita-city, 30-XI-2003, *H. Kashiwadani*, UPS L-170710; **Netherland**: Drente: Beilen, Holthe 21, 4-VI-1998, *A. Aptroot*, H; Noord-Brabant: Budel-Dorplein, 30-IV-1989, *A. Aptroot*, H; Budel-Dorplein, 3-X-1999, *A. Aptroot*, H; Utrecht: Leusden, Leusderheide, Jannetjesdal, 1-XII-2001, *A. Aptroot*, H; Near Soesterberg, along higway A28, 12-XII-1999, *A. Aptroot*, H; Fort Ruigenhoek on paht, 5-III-2009, *A. Aptroot*, Aptroot 68588; **Norway**: Oslo: Bekkelagshogda, Asliveien 7/9, 3-IX-2008, *T. Tonsberg*, BG L86605; **Pakistan**: Northwest Frontier: West od Pakistan, Sharhan, Kaghan Valley, 20-VIII-1959, *S. Ahomad*, L 794572; **Sweden**: Dalarna: Rättvik par., Tättviksheden, vid kalkverket, 4,1 Km ONO Rättvik, 22-IX-2000, *J. Hermansson*, UPS L-157808; Närke: Svennevad, 11-III-1951, *G. Kjellmert*, MA-lichen 960; **Slovakia**: *Kósnarová-Baua*, BRA-CR 10025; Slovenské rudohorie, Slovinky, VIII-1986, *I. Pisút*, BRA-CR 10044; Sñánke vrchy, Milic, Izra, 3 Km situ septentriosli, 10-VII-1978, *O. Chreño*, BRA-CR 10028; Bratislava: Malé Karpaty, Jur pri Bratislave, svah cesty nad Pustým kostolíkom, 18-IX-1971, *A. Lackoviová*, BRA-CR 10017; Planitia Záhorská nízina, Malacky, 27-VIII-1957, *I. Pisút*, BRA-CR 6108; Plavecké Podhradie, 17-IV-1969, *E. Jelínková*, BRA-CR 10037; Záhorská nizina, 11-VIII-1965, *L. Opold*, BRA-CR 10022; Záhorská nizina, ad viam a Borský Mikulás versus, VII-1978, *I. Pisút*, BRA-CR 10008; Záhorská nizina, Plavecký Stvrtok, 11-VIII-1965, *I. Pisút*, BRA-CR 10021; Záhorská nizina, Sastínske Stráze, 23-III-1971, *I. Pisut*, BRA-CR 10014; Záhorská nizina, Sastínske Stráze, Sastínske Stráze, 29-III-1971, *I. Pisút*, BRA-CR 10018; Záhorská nizina, Sastínske Stráze, Sastínske Stráze, 29-III-1971, *I. Pisút*, BRA-CR 10009; Záhorská nizina, Sastínske Stráze, Sastínske Stráze, 29-III-1971, *I. Pisút*, BRA-CR 10004; South Moravia: Znojmo, 27-XII-1949, *J. Komárek*, BRA-CR 5991; Trencin: Tribecske vrchy, Jlizské, 17-V-1988, *I. Pisút*, BRA-CR 10005; Zilina: Montes Západné Tatry, 18-VII-1958, *I. Pisút*, BRA-CR 6069; Podunajská nízina, X-1973, *I. Pisút*, BRA-CR 10010; Západné Tatry, Konská, 24-X-1984, *I. Pisút*, BRA-CR 10006; Carpates, montes Slovenské Rudohorie, Mnísek, in valle rivi Smolník prope Smolnická pila, 21-VII-1974, *A. Kiszely et A. Vezda*, BRA-CR 10033; **Spain**: Asturias: Pola de Laviana, 7-VIII-2003, *A. R. Burgaz*, MACB 96232; Barcelona: El Brull, Parc Nat. del Montseny, coll de Formic, 16-VIII-2006, *A. R. Burgaz*, MACB 94414, MACB 100473, MACB 94453; Gerona: El Masos de Pals, El Baix Empordan, 10-IX-2007, *A. R. Burgaz*, MACB 95955; Port de la Selva, L'Alt Empordan, San Pere de Rodes, 4-IX-2007, *A. R. Burgaz*, MACB 95957; Ridaura la Plana, 31-XII-1991, *A. Herrero*, MACB 45965; Sant Sadurní d'Osormort, La Selva, 15-IX-2007, *A. R. Burgaz*, MACB 95959; Lerida: Montellá, Sª de Cadí, camino al refugio de Prat d'Aguiló, 2-VII-1996, *A. R. Burgaz & I. Martínez*, MACB 96789; Navarra: Elzaburu, 9-IX-1991, *T. Ahti & A. R. Burgaz*, MACB 45966; Tarragona: Vilanova de Prades, Sª de Prades, 28-VII-2003, *A. R. Burgaz*, MACB 93801; Canary Islands: Isla de Hierro, III-1980, *G. Follmann & C. Hernandez-Padrón*, BRA-CR 1940; **Sweden**: Sverige: Gästrikland, Hille sn, Trödje detta Trödje station, vid byggnad, V-sidan, 2-VIII-2004, *G. Odelvik & B. Hellström 04786*, S F52894; **United States**: Massachusetts: Plymouth Co., Hingham, Boston Harbor Island National Park Area, Regged Island, north side od the island, 20-VIII-2001, *S. LaGreca*, FH; Suffolk Co., Boston, Boston Harbor Island National Park Area, Rainsford Island, 24-VIII-2001, *D. Greene*, FH; Minnesota: Lake County, Superior National Forest, 8 mi E

of Babbitt, large odl gravel pit W of Stony River surrounded by quaking aspen, 10-VIII-2004, *C. M. Wetmore No. 92312*, S F53070; Lake County, Superior National Forest, 8 mi E of Babbitt, large odl gravel pit W of Stony River surrounded by quaking aspen, 10-VIII-2004, *C. M. Wetmore No. 92312*, FH 259632.

***Cladonia scabriuscula* (Delise) Nyl.**

Argentina: Neuquén: Nahuel Huapi National Park, puerto Blest, Laguna Los Canteros, 30-X-1983, *L. Hämet & T. Ahti*, H; **Canada**: Newfoundland: Island of Newfoundland, Avalon Peninsula, Burry Heights Center, 7-IX-2007, *J. C. Lendemer*, H; Nova Scotia: Colchester Co., Portapique Wilderness Area, N of Montrose, E of Portapique River, 17-V-2004, *T. Ahti*, H; Cumberland Co., Cape Chignecto Provincial Park, 2-3 km NW of West Advocate, along the trail from Red Rocks of McGahey Brook, 15-V-2004, *T. Ahti*, H; **Chile**: Región XII, Magallanes y Antártida: Isla de Navarino, caleta Honda, pasado el puerto Navarino, 13-I-2005, *R. Vilches*, MACB 91976; Valparaiso: Juan Fernández Island, Masatierra, subida al mirador de Silkirk desde Sn. Juan Bautista, 27-I-1980, *O. Parra*, H; Juan Fernandez islands, Robinson Crusoe Island, subida al mirador Selkirk desde Sn. Juan Bautista, 27-I-1980, *O. Parra*, H; Juan Fernandez, Masatierra, Puerto Inglés, lado sur con vista hacia fuera, 31-I-1980, *O. Parra*, H; Juan Fernandez, Masatierra, subida al mirador de Selkirk desde Sn. Juan Bautista, 27-I-1980, *O. Parra*, H; **China**: Sichuan: NW Sichuan, Hongyuan Co. Shuan-Jin Temple, Si-Da-Lang-Gou at 150 Km, 22-IX-1991, *T. Koponen*, H; NW Sichuan, Minschan Range, Songpan Co. Huanglong Temple, 11-IX-1991, *T. Koponen*, H; NW Sichuan, Minshan Range, Namping Co., Jiu-Zhai-Gou, Ri-Ze-Gou, Natural Forest Reserve, 16-IX-1991, *T. Koponen*, H; **India**: Sikkim: East Sikkim district, Rechala surroundings, 15-IV-1996, *G. P. Sinha*, H; North Sikkim district, Yumthang along river side, 13-VII-1996, *G. P. Sinha*, H; West Sikkim district, Karchi Reserve forest, 15-XII-1994, *G. P. Sinha*, H; West Sikkim district, Yoksum-Tsoka track, 11-V-1994, *G. P. Sinha*, H; **Japan**: Kyushu: Hyuga (Pref. Miyazaki), Kirishima-Yaku National Park, Kirishima Mts., Mt. Karakunidake, 17-IX-1972, *T. Ahti*, H; **Nepal**: Eastem region: Mechi, De Bilbatay Banjyang á Hile, region de Chiltray, 11-X-1971, *P. Ozenda*, H; Mechi, De Telok et Tapletok, 7-X-1971, *J. F. Dobremez*, H; **Russia**: Khabarovsk Krais: Primor'e territory, Southern Sikhote-Alin Range, Sudzykhe State Reserve, 16-VII-1944, *P. Usudova & Pokrovskaya*, H; **Spain**: Asturias: Pajares, Puerto de Pajares, 22-VII-2007, *A. R. Burgaz*, MACB 95518; Ávila: Candelario, P. Nat. Sª de Candelario, subida a El Travieso, 1-VII-2007, *A. R. Burgaz*, MACB 95828; Segovia: El Espinar, valle del río Moros, área recreativa la Panera, 26-I-2008, *A. R. Burgaz*, MACB 96728; **Taiwan**: Hau-lien Co., Shyu-lin village, between Ta-yu-lin and Mt. Ho-huan, 23-X-1970, *T. Koponen*, H; Southwestern Taiwan: Chia-yi Co., Mt. A-li, 13-X-1970, *T. Koponen*, H; Chia-yi Co., Mt. A-li, 13-X-1970, *T. Koponen*, H; **Thailand**: Shan Highland: Doi Inthanan National Park, 31-III-2005, *S. Paonmen*, H; **United States**: Alaska: Seward Peninsula, 15-VII-2000, *S. Walker*, H; Seward Peninsula, Glacier Creek, 16-VII-2000, *S. Walker*, H; Seward Peninsula, Mauze Creek, 25-VII-2000, *S. Walker*, H; Tigalda Bay, Tigalda Island, Aleutian Islands, 13-VIII-2008, *S. S. Talbot & W. B. Schofield*, H; California: Riverside County, San Mateo Wilderness area, Los Alamos Canyon, 31-XII-2003, *K. Knudsen*, H; Maine: Washington co., on cliff overlooking Bay of Fundy Cobcook Bay State Park, Edmunds Township, 30-VIII-1995, *S. Hammer*, H.

***Cladonia stereoclada* Abbayes**

Portugal: Azores Islands: Terceira, Canada dos Pomares-Terra-Cha, 7-XI-2004, *A. F. Rodrigues*, H; Sao Miguel, Serra Aqua de Pau, Heide unter Passtrasse, Rib. Grande, 26-VII-2003, *F. Berger*, H; São Miguel, Furnas, Salto Cavallo, 200 m S. Abzw. Nach Povacao, 28-VII-2003, *F. Berger*, H; Mata da Esperanca, 2003, *A. F. Rodrigues*, H; Monte Brasil, 2003, *A. F. Rodrigues*, H; Madeira Island: Portela, 26-V-1977, *H. Schindler*, H; Ribeiro Frio, 23-II-1989, *V. Haikonen*, H; Ribeiro Frio, 7-I-1974, *E. Väisälä*, H; **Spain**: Canary Islands: Tenerife, Mazico de Anaga, Pico del Ingles, 15-XII-2005, *P. Alanko*, H; NE de Santa Cruz de Monte de las Mercedes, El Bailadero, 13-II-1989, *P. Uotila & R. Lidberg*, H; Sª de Anaga, Las Casas de la Cumbre, 15-I-1985, *P. L. Nimis*, H; Mazizo de Anaga, El Bailadero, 8-I-1983, *P. Alanko*, H; Mirador del Ingles, V-1980, *Follmann*, H; Las Mercedes W del Pico del Ingles, 18-I-1968, *L. Holm-Nielsen, S. Jeppesen, S. Laegaard & K. Ras*, H; Santa Cruz de Tenerife, Roque del Agua, 5-III-1986, *T. Feuerer & N. Höhne*, H; IV-1980, *G. Follmann & S. Scholz*, H; Las montañas de Anaga, en el monte Pico del Ingles, 8-VII-1994, *A. Vezda & F. Ceni*, H; Las Mercedes, Monte de las Mercedes, hacia El Bailadero, 16-VI-2007, *A. R. Burgaz*, MACB 97913, MACB 97911.

***Cladonia subcariosa* Nyl.**

Czech Republic: Moravia: Konitz b. Znaim, Thayaabhang, fonniges, kurzgrafiges Steppenland, felten fruchtend, 7-V-1921, *A. Oborny*, H; Wald zw. Zuckerhandl und Klein-Tesswitz bei Zaim, 23-X-1923, *A. Oborny*, H; South Moravia: Granitztal bei Znaim, Mähren, 1920, *Suza*, H; Znaim, an zerklüfteten Felswänder an der alter Pöltenberger StraBe, 19-V-1921, *A. Oborny*, H; Znaim, Leskatal, Stipatrift hinter der Porzellanfabrik, 19-XII-1922, *A. Oborny*, H; **Germany**: Bohemia: ad terram nudam in locis apricis prope Mies, *J. Lukasch*, H; Ad terram nudam in locis apricis prope Mies, *J. Lukasch*, H; **Haiti**: Dept. De L'OUEST, Shada Station, 19-VII-1958, *C. M. Wetmore*, H; L'Ouest, 4-VII-1958, *H. A. Imshaug*, H; **Japan**: Honshu: Aki, Fujikane, Kimita-mura, Futami-gum, 20-V-1978, *T. Watanabe*, H; Hitachi, Shishikura, Dejima-mura, Niihari-gum, 10-II-1994, *H. Kashiwadani*, H; Ibaraki Pref. (Prov. Hitachi), Tsukuba, Amakubo, by National Science Museum herbarium building, Strrtside lawn, 18-XI-2002, *T. Ahti*, H; Kai, Lakeside of Saiko, Mt. Fuji, 11-IV-1977, *H. Kashiwadani*, H; Suruga (Pref. Shizuga), Fuiyoshida, Mt. Fuji, 1968, *M. Togashi*, H; **New Zealand**: South Island: Canterbury, Boyle River 0.5-1 Km N of Boyle River Alpine Lodge, 14-IX-1981, *T. Ahti*, H; **Portugal**: Madeira Island: Weg von Encumeada, Boca das Torrhinas, 12-V-2004, *F. Bergen*, H; **Russia**: Sakha Republic: Yyakutia, Khangalasskiy Dist., Elenka (Yelanskoye) ca. 5 Km WNW of village centre, 19-VIII-2005, *T. Ahti & P. A. Timofeev*, H; Yyakutia, Khangalasskiy Dist., Elenka (Yelanskoye) ca. 5 Km WNW of village centre, 19-VIII-2005, *T. Ahti & P. A. Timofeev*, H; Yyakutia, Khangalasskiy Dist., Elenka (Yelanskoye) ca. 5 Km WNW of village centre, 19-VIII-2005, *T. Ahti & P. A. Timofeev*, H; **South Africa**: Cape province: Wellington Division, Groenberg, IV-1995, *H. Stadion*, H; **Sweden**: Götaland: Bohuslän, Norum, Stora Askerön, 19-VII-1948, *A. H. Magnusson*, H; Bohuslan, Steömstad, Rossö W, North of Rossönäs, 9-IV-2005, *B. P. Lofall*, H; Bohuslan, Steömstao, Rossö NW, near Ölbergsholmen, 9-IV-2005, *B. P. Lofall*, H; **Switzerland**: Zürich: Auf trockenem Trofland bei Riffersweil, Kanton Zürich, *Hegetschweiler*, H; **United States**: Alabama: Jackson Co., Flat Rock, NE of intersections, 2-X-1999, *T. Ahti*, H; Arkansas: Hot Springs National Park, Upper Floral Trail on Hot Springs Mt., 17-V-2001, *C. M. Wetmore*, H; Florida: Bradford County, Lake Butler Widlife Management Area, Raiford Tract Co. Rd. 125, 6.1 mile ENE of U.S., 3-XII-1994, *C. Harris*, H; Lousiana: Baton Rouge, Denham Springs, Livington, II-1972, *B. Exner*, H; Maine: Washington Co., on cliff overlooking Bay of Fundy, Cobscook Bay State Park, Edmunds Township, 30-VIII-1995, *S. Hammer*, H; Maryland: S of Whiteford, Quarry Road, Harford Co., 9-V-1983, *F. Reed*, H; New Jersey: Cumberland County, 1/2 mile west of intersection with May's Landing Road, ca. 1/2 mile east of intersection with Union Road, 8-VII-2003, *C. Lendemer, J. A. Macklin & G. Moore*, H; Cumberland County, 1/2 mile west of intersection

with May's Landing Road, ca. 1/2 mile east of intersection with Union Road, 8-VII-2003, *C. Lendemer, J. A. Macklin & G. Moore*, H; Monmouth Co., Gateway National Recreation Area, Sandy Hook, 6-V-1994, *T. Ahti*, H; Monmouth Co., Gateway National Recreation Area, Sandy Hook, 6-V-1994, *T. Ahti*, H; Ocean Co., 3 Km S of Warren Grove, 6-V-1984, *T. Ahti*, H; New York: Long Island, Suffolk Co., 2.5 Km of Riverhead, along Middle Country Road W of Northville Turnpike, 21-IV-1984, *T. Ahti*, H; Long Island, Suffolk Co., Promised Land, along Napeague Bay, S of Cranberry Hole Road, 21-IV-1985, *T. Ahti*, H; North Carolina: Bladen County, 17-VII-2002, *C. Lendemer, S. Hammer, J. Franklin, S. Herrera & S. Syed*, H; Johnston County, 13.5 Km ad occidentem versus a Smithfield, secus viam dictam Rt. 210, 9-III-1983, *W. L. Culberson & C. F. Culberson*, H; Johnston County, ad meridiem et occidentem versus ab Edmundson, secus viam dictam Rt. 210, 9-III-1983, *W. L. Culberson & C. F. Culberson*, H; Pender County, 8.6 mile NW of Hampstead, 22-IX-1968, H; Texas: Blanco County, Pedernales Falls State Park, 10 mils (16 Km) E of Johnson City, 28-X-1977, *R. S. Egan*, H; Hardin County, 4 mile (6.4 Km) SE of Saratoga, Big Thinker National Preserve, Lance Rosier Unit, 9-I-1976, *R. S. Egan*, H; Milam County, Sugar Loaf Mountain, 5 Km NNE of Gause, 14-X-1976, *R. S. Egan*, H; Orange County, 8 miles (12.8 Km) N of Beaumont off FM 1131; Big Thincker National Preserve, Beaumont Unit, 24-IV-1976, *R. S. Egan*, H; Wisconsin: Iowa, NB of Clyde on sand plains, 29-V-1968, *W. Thomson*, H; **Uruguay**: Rocha: Castillos, Camino Real, 18-V-2001, *G. Geymonat*, H; **Unknown**: Auf Frde alter Berghalden bei Mies in Böhmen, III-1897, *Lukasch*, H; Trockene StraBenböfchung oberhalb Mirotein b. Wenzelsdorf, Nordmähren, V-1917, *F. Schenk*, H.

***Cladonia subconistea* Asahina**

China: Chekiang (Zhejiang): Moganoshan, Wujitou, 7-IX-1980, *L. Hämet-Ahti*, H; Yunnan: Heqin Co., Song Guei village, Sclerophyll schrub, highway between Lijiang and Dali, 21-X-2002, *A. Aptroot*, H; Kunming Co., 5 Km W of Kunming, 16-X-2002, *A. Aptroot 55498*, H; **India**: Arunachal Pradesh: Debang Valley, Punli Mathuli foot track near Yarun, 1-II-1987, *K. P. Singh*, H; **Japan**: Honshu: Ibaraki Pref, Prov. Hitachi, Tsukuba, Amakubo by National Science Museum herbarium building, 18-XI-2002, *T. Ahti*, H; Ibaraki Pref, Prov. Hitachi, Tsukuba, Amakubo by National Science Museum herbarium building, 18-XI-2002, *T. Ahti*, H; Kyushu: Bungo (Pref.Ohita), Machida, Kokonoe-machi, Kusu-gum, 22-X-2002, *H. Kashwadani, Y. Umezu & K. Umezu*, H; Shinano: Takasawa, Shinanojiri, Kami-Minochi-gun, 4-X-1959, *M. Togashi*, H; **North Korea**: Däsongsan, N von Pyongyang, auf Wegabstich, 25-IX-1986, *S. Huneck*, H; Gyonggy: Near the Kyewon Art College, Mt. Morak, Naeson-dong, Uhwang city, 35-III-2003, *K. H. Moon*, H; **Taiwan**: Chia-yi Co., Mt., A-li, 13-X-1970, *T. Koponen*, H; Hua-lien Co., Shyu-lin Village, Tien-hsiang,garden of Tien-hsiang temple, 25-X-1970, *T. Koponen*, H; Nantou County: 25 Km ENE of Puli, Lushan, rodside, 13-X-2001, *A.Aptroot*, H; 45 Km WN of Hualien, Meifeng, around field centre, 9-X-2001, *A. Aptroot*, H; Taipei: Yangmingshan, 5-V-1975, *J. R. Wang-Yang*, H; Yangmingshan, 5-V-1975, *J. R. Wang-Yang*, H.

***Cladonia subrangiformis* Sandst.**

Bosnia Herzegovina: Sarajevo: Trevici, 28-III-2010, *A. R. Burgaz*, MACB 101105; **China**: Xinjiang province: Tianshan Mountains, Shawan, 20-VII-2000, *A. Abbas*, FH; **Denmark**: Zealand: Møn, E of Busemarke, Höyblege, 1-VI-1969, *T. Ahti*, H; **Estonia**: Harju district, Kostiverdi State Farm (20 Km E of Tallin), 1-VI-1976, *T. Ahti*, H; **France**: Hérault: Juvignac near Montpellier, Caunelle, 1-IV-1959, *A. Touw*, L 794557; **Germany**: Baden-Wurtemberg: Kahlberg b. Wertheim, Baden, 1921, *A. Kneucker*, H; **Iran**: Azarbayejan: Tabriz, Jolfa toward Khoda afarin Missan village, 10-V-2003, *M. Sohrabi*, H; Azerbijan: Tabriz, Jolfa toward to Khodaafrim, Missan village, 15-VII-2001, *M. Sohrabi*, H; Tabriz, Jolfa toward to Khodaafrin, Missan village, around village and toward the forest part, 5-VI-2003, *M. Sohrabi*, H; Mazandaran: Nuor, Kojdur and Kodir villages, 3-IV-2002, *M. Sohrabi & M. Mofid*, H; **Italy**: Toscana: Monte Ferrató (Prato), 29-XI-2000, *R. Benesperi*, H; **Netherlands**: Limburg: T'Rooth, quarry, 3-V-2003, *A. Aptroot*, H; **Portugal**: Algarve: Alijezur, Praia do Monte Clérigo, 9-XII-2006, *A. R. Burgaz*, MACB 94406; Beira Litoral: Campises, Sª de Sicó, 29-I-1996, *A. R. Burgaz & I. Martínez*, MACB 66914, MACB 66913; Serra de Sicó, Campses, 29-I-1996, *A. R. Burgaz & I. Martínez*, MACB 91129; Madeira Island: Montagne de la Sérenne, Ranquas (South of Madeirés), 1991, *T. Borsch*, FR 52678; Trás Os Montes: Izeda, valle del río Sabor, *A. R. Burgaz*, MACB 102807; Izeda, valle del río Sabor, 5-IX-2006, *A. R. Burgaz*, MACB 102802; **Spain**: Alava: Leza, Sª de Cantabria, subida al Puerto de Herrera, 26-VII-2006, *A. R. Burgaz*, MACB 94380; Peña Cerrada, Sª de Cantabria, Puerto de Herrera, 26-VII-2006, *A. R. Burgaz*, MACB 93739; Tertanga, Puerto de Orduña, 25-VII-2006, *A. R. Burgaz*, MACB 93740; Almería: Cabo de Gata, Sª de Cabo de Gata, 9-X-2002, *A. R. Burgaz*, MACB 96027; Asturias: Somiedo, valle de Lago, 31-X-1993, *G. Aragón, A. Herrero & I. Martínez*, MACB 96510; Sotres, vega de Cuerres, 20-IX-2004, *G. Aragón & R. Belinchón*, MACB 102947; Ávila: Candeleda, Arroyo de Santa María, 19-III-1993, *I. Martínez*, MACB 91139; Peguerinos, campamento Peñas Blancas, *A. R. Burgaz*, MACB 102947; Barcelona: Between La Roca and Orrius, 15-IV-1977, *F. Adema*, L 794567; El Bages, Castellfollit del Boix, Sª de Rubio, 20-V-1998, *A. R. Burgaz*, MACB 91148; El Bages, puerto del Bruch, El Parrot, 20-V-1998, *A. R. Burgaz*, MACB 91149; L'Anoia, Carme, camino Les Esplugues, 20-V-1998, *A. R. Burgaz*, MACB 91145; Burgos: Atapuerca, 27-IX-1999, *A. R. Burgaz & I. Rodríguez de Lope*, MACB 91131; Ayoluengo, 23-VII-1999, *A. R. Burgaz*, MACB 91141; Covarrubias, Sª de Covarrubias, 6-IX-2003, *A. R. Burgaz*, MACB 91157; Cuestaedo, subida a las antenas, 25-VII-2006, *A. R. Burgaz*, MACB 93510, MACB 93741; Espinosa de Cervera, 21-VIII-2003, *A. R. Burgaz*, MACB 102842; Espinosa de Cervera, sabinares, *A. R. Burgaz*, MACB 102842; Huidobro, 7-VI-1988, *A. R. Burgaz & E. Fuertes*, MACB 44210; Páramo de Masa, 9-VI-1988, *A. R. Burgaz & E. Fuertes*, MACB 44211; San Martín de Humada, 10-VIII-1998, *A. R. Burgaz*, MACB 91161; Sargentes de la Lora, 23-VII-1999, *A. R. Burgaz*, MACB 91133; Sargentes de Lora, 9-IV-1988, *A. R. Burgaz & E. Fuertes*, MACB 44221, MACB 44207; Cantabria: Fombellida, 24-VII-2006, *A. R. Burgaz*, MACB 93742, MACB 93511; Castellón: Castell de Cabres, 25-VI-1992, *A. R. Burgaz*, MACB 102792; Frades, colonia Europa II, P. Nat. Els Port, 9-IV-2003, *A. R. Burgaz*, MACB 91144, MACB 91011; Ciudad Real: Villamanrique, estribaciones de Sª Morena, 6-II-2003, *A. R. Burgaz*, MACB 91015, MACB 91127; Cuenca: Campillo-Sª, Valdeliebres, 29-V-1996, *A. R. Burgaz & I. Martínez*, MACB 91150; Uña, Ciudad Encantada, 14-IV-1990, *A. R. Burgaz*, MACB 37090; Gerona: San Martín de Ogassa, mirador coll de la Torre, 17-VIII-2006, *A. R. Burgaz*, MACB 94376; Guadalajara: Alcolea, 8-VI-1971, *R. Carballal*, MACB 66255; Almiruete, 7-XII-2001, *E. Ron & T. Ballesteros*, MACB 84691; Cabrera, 20-IV-1991, *A. R. Burgaz*, MACB 44217; Fuencemillán, 4-V-2005, *A. R. Burgaz*, MACB 93016; Jadraque, 14-IV-2006, *R. Pino-Bodas*, MACB 102858, MACB 102859; La Fuensaviñán, 12-X-2002, *A. R. Burgaz*, MACB 102793; Luzón, 11-VI-1992, *A. R. Burgaz & E. Fuertes*, MACB 44220; Monte de la Alcarria, *Mª E. Ron*, MACB 66256; Monte de la Alcarria, 17-VI-1070, MACB 5113; Monte de la Alcarria, 18-II-1971, MACB 5114; Muduex, 22-IV-2007, *A. R. Burgaz*, MACB 95414; Negredo, 21-IV-1991, *A. R. Burgaz*, MACB 44213, MACB 44214; Pelegrina, 20-IV-1991, *A. R. Burgaz*, MACB 44218; Tordesilos, 11-VI-1991, *A. R. Burgaz & E. Fuertes*, MACB 44219; Torija, 26-X-1990, *A. R. Burgaz*, MACB 44212; Zaorejas, 2-VI-2003, *A. R. Burgaz*, MACB 91162; Huesca: Arguis, Parque de Guara, Sª de la Gabardiella, antenas de TVE, 22-VIII-2006, *A. R. Burgaz*, MACB 94377, MACB 94738; Escalona, Urbez, valle del río Vellos P. Nat. de Ordesa y Monte Perdido, 28-VII-1998, *A. R. Burgaz*, MACB 91146; La Rioja: Bobadilla-Villaverde de Rioja,

Montes de Suso, 9-IX-2004, *A. R. Burgaz*, MACB 89573; Lumbreras, 22-X-1983, *A. R. Burgaz & Mendiola*, MACB 37089; Lérida: Espot, Pista de Lladres, ríu de Peguera, 12-VII-1994, *G. Aragón, J. Castillo & I. Martínez*, MACB 91125; La Vansa-Fornols, Ges, Sª del Cadí, 3-VII-1996, *A. R. Burgaz & I. Martínez*, MACB 102804; Madrid: Belmonte de Tajo, 20-XI-1998, *A. R. Burgaz*, MACB 68536; Buitrago de Lozoya, 26-XI-1989, *A. R. Burgaz*, MACB 37087; Bustarviejo, Puerto de Canencia, arroyo del Sestil del Mahillo, 30-I-1994, *G. Aragón & I. Martínez*, MACB; Colmenar de Oreja, finca Encomienda Mayor de Castilla, *A. R. Burgaz*, MACB 102831; Miraflores de la Sª, subida al Pto. de Canencia, suelo ácido, *A. R. Burgaz*, MACB 102837; Embalse de Riosequillo, 21-III-1997, *A. R. Burgaz & S. Casas*, MACB 75236; Galapagar, 4-XII-1989, *A. R. Burgaz*, MACB 37088; Manzanares el Real, P. Nat. de La Pedriza, senda de Quebrantaherraduras, *A. R. Burgaz*, MACB 93009; Montejo de la Sª, 8-III-2003, *P. Aguilar, G. Amo de Paz & A. R. Burgaz*, MACB 91128; Morata de Tajuña, 20-III-1993, *I. Acebal*, MACB 102899; Puerto de Canencia, 14-XI-1995, *A. R. Burgaz*, MACB 102840; Puerto de Canencia, 22-IV-1996, *A. R. Burgaz*, MACB 102835; Redueña, 11-V-1998, *A. R. Burgaz*, MACB 102832; Redueña, 13-V-1994, *A. R. Burgaz*, MACB 91160; San Martín de la Vega, carretera a Morata de Tajuña, 10-VI-1993, *G. Aragón, J. Castillo & I. Martínez*, MACB 91124; Torrelaguna, 18-V-1994, *A. R. Burgaz*, MACB 102841; Murcia: Zaén de Arriba, 10-IV-2001, *A. R. Burgaz*, MACB 91126; Navarra: Elzaburu, 9-IX-1991, *T. Ahti, A. R. Burgaz & E. Fuertes*, MACB 44227; Foz de Arbayún, 01-XI-2003, *A. R. Burgaz*, MACB 91152; Palencia: Areños, Puerto de Piedras Luengas, 12-IV-1991, *A. R. Burgaz & N. Marcos*, MACB 44222; Resoba, Reserva Nacional de Fuentes Carrionas, 24-VII-2006, *A. R. Burgaz*, MACB 94379; Villaviudas, 16-IV-1984, *A. R. Burgaz*, MACB 10813; Villaviudas, 28-IV-1983, *A. R. Burgaz*, MACB 13037; Salamanca: Fuenterroble de Salvatierra, 5-XI-2005, *A. R. Burgaz*, MACB 93022; Fuenterroble de Salvatierra, Sª de Frades, 5-XI-2005, *A. R. Burgaz*, MACB 102819; Segovia: Cedillo de la Torre, 30-IV-1984, *A. R. Burgaz & M. Ventureira*, MACB 14195, MACB 17216, MACB 17211, MACB 14194; El Espinar, valle del río Moros, área recleativa la Panera, 26-I-2008, *A. R. Burgaz*, MACB 102799; La Granja, Valsaín, río Eresma, fuente de los dos caños, 26-I-2008, *A. R. Burgaz*, MACB 102800; Montejo de la Vega de la Serrezuela, cañón del río Riaza, 27-IX-1992, *A. Herrero*, MACB 91014; Revenga, cordel de Peñas Zamarriegas, 26-I-2008, *A. R. Burgaz*, MACB 96760, MACB 102798; Soria: Almazán, 6-IV-1993, *A. R. Burgaz*, MACB 102791; Alto de Villaciervos, 10-IX-1991, *T. Ahti, A. R. Burgaz & E. Fuertes*, MACB 44225, MACB 44226; Alto de Villaciervos, 21-IX-1990, *A. R. Burgaz*, MACB 44206; Alto de Villaciervos, La Fragua, 31-V-1999, *A. R. Burgaz, M. A. Carrasco & E. Fuertes*, MACB 91158; Berlanga de Duero, 13-IV-2006, *R. Pino-Bodas*, MACB 102883; Lubia, Altos de Lubia, 7-IX-2003, *A. R. Burgaz*, MACB 102819; MACB 91012; Matalebrera, 10-IX-1991, *T. Ahti, A. R. Burgaz & E. Fuertes*, MACB 44231, MACB 91155; Puerto de Santa Inés, vertiente norte, 7-IX-2003, *A. R. Burgaz*, MACB 102823; Villaciervos, 20-IV-1993, *A. R. Burgaz*, MACB 91154; Tarragona: Col de Guá, 25-VI-1992, *A. R. Burgaz*, MACB 91153; Conca de Barberá, Prades, granitos, *A. R. Burgaz*, MACB 102838; Rasquera, Sª de Cardó, 9-IV-2003, *A. R. Burgaz*, MACB 91137, MACB 91147; Refalgueri, P. Nat. Els Ports, 9-IV-2003, *A. R. Burgaz*, MACB 91140; Vilanova de Prades, Sª de Prades, 28-VII-2003, *A. R. Burgaz*, MACB 91138; Teruel: Albarracín, Sª de Albarracín, barranco del Navazo, 15-V-1996, *G. Aragón, A. Herrero, I. Martínez*, MACB 102900; Cumbre de Javalambre, 14-VI-1991, *A. R. Burgaz & E. Fuertes*, MACB 44229; Ejulve, llano de Villaseco, 24-VI-1992, *A. R. Burgaz*, MACB 91159; El Parrisal, Beceite, P. Nat. de Los Puertos de Beceite, río Matarraña, 8-IV-2003, *A. R. Burgaz*, MACB 91136; Escucha, Puerto de San Just, 23-VI-1992, *A. R. Burgaz*, MACB 96029; Fonfría, Sª de Cucalón, 23-VI-1992, *A. R. Burgaz*, MACB 96028, MACB 95956; Frias de Albarracín, 12-VI-1991, *A. R. Burgaz & E. Fuertes*, MACB 44228; Llano de Villaseco, 24-VI-1992, *A. R. Burgaz*, MACB 91130; Noguera, Sª de Albarracín, barranco de los Polos, 18-V-1996, *A. R. Burgaz*, MACB 102836; Puerto de Valdecuenca, 12-VI-1991, *A. R. Burgaz & E. Fuertes*, MACB 44223, MACB 44315; Puerto de Valdelinares, Sª de Gudar, 13-VI-1991, *A. R. Burgaz & E. Fuertes*, MACB 44230, MACB 44224; Puerto Majalinos, 23-VI-1992, *A. R. Burgaz*, MACB 91156; Puerto Sant Just, 23-VI-1992, *A. R. Burgaz*, MACB 91151; Subida al puerto de Javalambre, 14-VI-1991, *A. R. Burgaz & E. Fuertes*, MACB 44216; Villar del Cabo, 12-VI-1992, *A. R. Burgaz & E. Fuertes*, MACB 44209; Toledo: Urda, Sª Morrones, subida antena TV, 5-XI-2004, *A. R. Burgaz*, MACB 102824; Valencia: Cofrentes, 28-V-1996, *A. R. Burgaz & I. Martínez*, MACB 91143; Valladolid: Portillo, 27-X-1984, *A. R. Burgaz*, MACB 37087; Santa Espina, 1-IX-2000, *A. R. Burgaz*, MACB 102821, MACB 91142; Urueña, 20-VII-2006, *A. R. Burgaz*, MACB 93512, MACB 93743, MACB 93744; Zamora: Villalazán, 6-X-1997, *G. Aragón, A. R. Burgaz & A. Terrón*, MACB 70859, MACB 70222; Villalazán, 7-IX-1998, *A. R. Burgaz, S. Casas & I. Rodríguez de Lope*, MACB 70221; Zaragoza: Pina de Ebro, 7-XII-2007, *A. R. Burgaz*, MACB 96291; Canary Islands: El Hierro, Frontera, P. Nat., 5-X-2001, *N. Marcos & D. Manso*, MACB 91132; La Coruña: Muros, playa de Lariño, 2-IX-2002, *A. R. Burgaz*, MACB 91134, MACB 91135; Baleares Islands: Mallorca, Porto Cristo, Cuevas del Drach, 15-IV-1997, *E. Ortega & P. Navarro*, MACB 64903; **Sweden**: Öland: Böda, Mensalvaret, 3-VIII-1983, *O. Vitikainen*, H; Mörbylanga comun and socken, Möckelbossen S, 8-VI-2006, *P. Alanko*, H; Resmo par., 1.5 Km E Resmo just N of the road to Stenåsa, 12-VI-1986, *M. Wedin*, UPS L-08681; S. Möckleby par., Gettlinge alvar ca 400m E the road from Gårdstorp, 11-VI-1986, *M. Wedin*, UPS L-08689; Torslunda alvaret, 8-VII-1948, *G. Kjellmert*, H; Torslunda, alvaret, 8-VII-1948, *G. Kjellmert*, H; **Turkey**: Zonguldak: Beycuma County, Cayköy district and surrounding, 20-VIII-2005, *K. Yazici*, H; **Ukraine**: Ditio Kioviensis, Perejaslaw-Chmelnickij, 27-VIII-1924, *A. Oxner*, H; **United Kingdom**: England: Gloucestershire, 22-X-1966, *D. L. Hawsworth*, H; Surrey, Banstead, Banstead Downs, 17-VII-1969, *T. Ahti & J. R. Laudon*, H; **Unknown**: 1868, *Dr. Rehm*, FR 75455; Causse du Larzac, Rand der Hochfläche zur Vis-Schlucht nördl. St. Maurice de Navacelles, 5-X-1993, *T. Borsch*, FR 52683.

***Cladonia subturgida* Sampaio**

Material estudiado enumerado en el ARTÍCULO II

***Cladonia subulata* (L.) Weber ex F. H. Wigg.**

Andorra: Les Escaldes-Engordany, bajando del collado de Jovell hacia el refugio Fontverd, valle del río Madriu, 6-XII-1994, *A. Herrero & I. Álvarez-Fernandez*, MACB 64884, MACB 64885; Ordino, coll de Ordino, 19-VII-2006, *A. R. Burgaz*, MACB 94814; Vall d'Incles, río de Juclar, subida al refugio de Sisqueró, 3-VII-1996, *A. R. Burgaz & I. Martínez*, MACB 96674, MACB 95097; Vall d'Incles, subiendo hacia el refugio de Sisqueró, 7-XII-1994, *A. Herrero & I. Álvarez Fernández*, MACB 64883; **Austria**: Bez. Schärding, Kopfing, Steinbruch Schnürberg, 26-IV-2009, *F. Berger* 23733; **Australia**: New South Wales: Vicinity of Clyde Mountain, ca. 20 Km SE of Braidwood, 9-VII-2000, *S. Hammer*, FH; Southrem Tableland, vicinity of Clyde Mountain, ca. 20 Km SE of Braidwood, 9-VIII-2000, *S. Hammer*, CANB 654473; Queensland: Binna Burra, Lamington National Park, VII-2000, *S. Hammer*, FH; Moreton, Binna Burra, Lamington National Park, VII-2000, *S. Hammer*, CANB 654482; **Belgium**: Liège: Liège, Sprimont, Gem. Spimont, ± 1 Km ten Z. van Higné [Ligné?], 23-IV-1973, L 229894; **Chile**: Región XII, Magallanes y Antártida Chilena: Isla de Navarino, caleta Honda, pasado Puerto Navarino, 13-I-2005, *R. Vilches*, MACB 92217; Isla de Navarino, caleta Wulaia-Puerto Inutil, 26-I-2005, *R. Vilches*, MACB 92158; Isla de Navarino, Puerto Williams, valle del río Ukika, camino a Media Luna, 18-I-2005, *A. R. Burgaz*, MACB 92215; Isla de Navarino, Puerto

Williams, valle del río Ukika, 17-I-2005, *A. R. Burgaz*, MACB 92218; Isla de Navarino, caleta Wulaia, 23-I-2005, *R. Vilches*, MACB 92081; Isla de Navarino, Lago Róbalo, 18-I-2005, *R. Vilches*, MACB 92080; Isla se Navarino, camino de Lum, 26-I-2005, *R. Vilches*, MACB 92216; **Czech Republic**: Central Bohemian: Krivoklátsko, Krivoklat, Kalubice, 4-XI-2000, *J. Vondrák*; Sandiger Heideboden b. Rehdörfel n. B-Leipa, 1930, *J. Anders*, BRACR 10056; Moravia: Brno, supra Závist prope Cerná Hora, 11-II-1973, *A. Vezda*, BRACR 10036; Distr. Brno, supra pagum Mokrá Hora, VI-1971, *A. Vezda*, BRACR 10030; Tisnov, 8-V-1971, *A. Vezda*, BRACR 10048; South Bohemiam: Trebonská pánev basin, Veselí n/Luz, Vlkovská pískovna, 12-X-200, *J. Vondrák*; Ceské Budejovice, Nové Hodejovice,damm of setting pit above village, 2-IV-2009, *J. Vondrák* 6983; Slavkovský les upland: Sokolov, Krásno 18-IV-2009, *J. Voldrák* 7054; **Denmark**: Hovedstaden: North Sealand, Hillerod, Tisvildeleje, woodland area Tisvilde SW of town, 14-III-2009, *J. Vondrák* 6967; Midtjylland: Velling Koller N. Bryrup, 12-V-1984, *S. Svane*, L 794580; **Finland**: Uusima: Sibbo, Skogsby Storstenkläven, 20-VI-1990, *T. Ahti, A. R. Burgaz & F. Fuertes*, MACB 50754; Nurmijärvi, Perttula, Äijänkallio, 29-IX-1995, *T. Ahti, A. R. Burgaz, I. Martínez & O. Vitikainen*, MACB 96315; **France**: Aquitaine: Frankrijk Dép. Gironde, Meer van Lacau, lanf Canal du Porge, ten Zuiden van het meer, tussen gras en struiken, op Zand, 19-VII-1971, *N. V. Donkersfoed*, BRACR 10023; Auvergne: Cantal Albepierre, near Murat, 26-VII-1998, *L. Spier*, L 752940; Midi Pyrénées: Lot Vers, 13-VII-2000, *L. Spier*, L 753001; Nord-Pas-de-Calais: Fourmues, 12-IV-1970, *J. Lambinon*, BRACR 10049; **Germany**: Bavaria: Bürgerwald b. Mähr-Schönberg, 1930, *F. Schenk.*, BRACR 10057; North Rhine Westphalia: Brandenbrg, Brandenderg, XI-2002, *P. Thomas*, L 668676; **Netherland**: Gelderland: Betuwe, Bergharen, De Berg, 15-III-2001, *A. Aptroot*, H; Veluwe, Heerde, Kamperklippen and Tonnenberg, 2-IV-1999, *A. Aptroot*, H; Noor-Brabant: Budel-Dorplein, 3-X-1999, *A. Aptroot*, H; Utrecht: Leuseden, along Leusdense Pad, III-1990, *L. Spier*, BRACR 10038; Soest, De Stompert, 15-XI-1998, *A. Aptroot*, H; De Leusderheide, Leusden, III-009, *L. Spier*, Spier 17773; De Stompert, Soest, III-009, *L. Spier*, Spier 17774; Den Treeh, Leusden, III-009, *L. Spier*, Spier 17717; Den Treek, Leusden, III-009, *L. Spier*, Spier 17772; **New Zealand**: South Island: Maitai Bush Trail, vicinity Nelson, XII-1998, *S. Hammer*, FH; Tantagree Forest Track, vicinity of Nelson, XII-1998, *S. Hammer*, CANB 653734; Tantagree Forest Track, vicinity of Nelson, XII-1998, *S. Hammer*, FH; **Portugal**: Algarve: Monchique, Sª de Monchique, subida a Foia, 8-XII-2006, *A. R. Burgaz*, MACB 93791; Alto Alentejo: Montinho, P. Nat. São Mamede, 11-I-2004, *A. R. Burgaz*, MACB 96243; P. Nat. São Mamede, subida al pico Sao Mamede, 10-I-2004, *A. R. Burgaz*, MACB 96236; Beira Alta: Castelo Mendo, 1-V-1997, *A. R. Burgaz*, MACB 94885; Manteigas, Sª da Estrela, P. Nat. de Sª da Estrela, cabecera del río Zêzere, 2-V-2007, *A. R. Burgaz*, MACB 94837; Manteigas, Sª da Estrela, P. Nat. de Sª da Estrela, poço do Inferno, 2-V-2007, *A. R. Burgaz*, MACB 94886; Estrela, Sª da Estrela, ribeira da Pragueira, 22-X-1995, *N. Marcos, E. Munin & P. Navarro*, MACB 64882; Penhas Douradas, Sª da Estrela, P. Nat. de Sª da Estrela, 2-V-2007, *A. R. Burgaz*, MACB 94836, MACB 66939; Sabugueiro, Lagoa Comprida, Sª da Estrela, P. Nat. de Sª da Estrela, 3-V-2007, *A. R. Burgaz*, MACB 95830; Valle de Amoreira, Sª da Estrela, P. Nat. de Sª da Estrela, subida a Quinta do Fragusto, 2-V-2007, *A. R. Burgaz*, MACB 95092; Beira Litoral: Luso, Bussaco a 2 Km de Cruz Alta, 3-IV-1998, *T. Ahti & A. R. Burgaz*, MACB 66935; Sª do Açor, Fraga de Pena, Barroco de Degrainhos, 1.5 Km SSE de Benfeite, 29-III-1998, *T. Ahti & A. R. Burgaz*, MACB 66936; Sª do Açor, Fraga de Pena, Barroco de Degrainhos, 1.5 Km SSE de Benfeite, 29-III-1998, *T. Ahti & A. R. Burgaz*, MACB 66938; Sª do Açor, Mata de Margaraça 2.5 Km de Benfeite, 29-III-1998, *T. Ahti & A. R. Burgaz*, MACB 66937; Estremadura: Azoia, Sª de Sintra, 10-I-2004, *A. R. Burgaz*, MACB 96242; Trás Os Montes: Alimonde, Sª da Nogueira, valle de Alimonde, 6-IX-2006, *A. R. Burgaz*, MACB 93997; Alrrededores de la Sª de Nogueira, 20-II-2005, *A. R. Burgaz*, MACB 95241; Izeda, valle del río Sabor, 5-IX-2006, *A. R. Burgaz*, MACB 93965; Lagoa, valle del río Sabor, 5-IX-2006, *R. Pino*, MACB 93692, MACB 93996; Rebordainhos, Sª de Nogueira, 19-II-2005, *A. R. Burgaz & J. Marques*, MACB 94710; Rebordaos, 12-VIII-1994, *A. R. Burgaz*, MACB 97778; **Slovakia**: distr. 13a, 16-V-1969, *S. Svane*, BRACR 10050; S of Skive, distr. 15, *S. Svane*, BRACR 10051; Bonská Bystinca: Kremnické pohorie, montis Krahulský Stos, Kremnica, 25-IV-1970, *I. Pisút*, BRACR 10007; Kremnické pohorie, montis Krahulský Stós supra opp. Kremnica, 25-IV-1970, *I. Pisút*, BRACR 10015; Kremnické, in decl. inter montes Kalvária et Sturec, *I. Pisút*, BRACR 10045; Montes Muránska planina in valle Hrdzavá dolina, 23-VII-1960, *I. Pisút*, BRACR 6092; Sliac, Ovsemno, 21-VIII-1952, *J. M. Novacký*, BRACR 10029; Kosice: Brdy, in monte Plesivec supra pag. Rejkovice, 14-VIII-1940, *H. Sandstede*, BRACR 10013; Košice: Slánske vrchy, Milic, Izsa, 8-VII-1978, *O. Chreño*, BRACR 10040; Prešov: Slavkovský les upland, Sokolov, Horní Slavok, settling pit c. 1km S of town, 18-IV-2009, *J. Vondrák*, ; Slavkovský les upland, Sokolov, krásmo, destroid peat-bog c. 2 Km W of village, 18-IV-2009, *J. Vondrák*; Vysoke Tatry, 21-VIII-1972, *L. Opold*, BRACR 10047; Vysoké Tatry, 27-VIII-1972, *L. Opold*, BRACR 10046; Vysoké Tatry, Strbské, XI-1965, *L. Opold*, BRACR 10039; Západne Slovensko: Bratislava, Bohemoslovacia, BRACR 10027; Západne Slovensko: Bratislava, Bohemoslovacia, BRACR 10035; **Spain**: Asturias: Felechosa, 5-VIII-2003, *A. R. Burgaz*, MACB 95295; Felechosa, Foces del Pino, 5-VIII-2003, *A. R. Burgaz*, MACB 100478; La Raya, Reserva Nac. de Mampodre, Puerto de San Isidro, 22-VII-2006, *A. R. Burgaz*, MACB 93151; Pola de Lena, 7-VIII-2003, *A. R. Burgaz*, MACB 95296; Rengos, Puerto de Rañadoiro, 24-X-1994, *I. Martínez & A. R. Burgaz*, MACB 64878; Vega de Espinareda, Burbia, 13-VIII-1994, *I. Martínez*, MACB 64879; Ávila: Bartolomé de Bejar, 1-VII-2007, *A. R. Burgaz*, MACB 95336; Candelario, P. Nat. Sª de Candelario, subida a El Travieso, 1-VII-2007, *A. R. Burgaz*, MACB 95337; Casavieja, Fuentelechada, 9-XI-2003, *A. R. Burgaz*, MACB 93837; El Raso, hacia el pico Almanzor, 16-II-2006, *R. Pino-Bodas*, MACB 96017; Hoyocasero, 22-VIII-2001, *A. R. Burgaz*, MACB 95098; Navacepedilla de Corneja, 21-XI-2004, *A. R. Burgaz*, MACB 95155; Navalperal de Tormes, P. Nat. de Sª de Gredos, subida a la laguna Grande, cuerda del Cuento, 30-VI-2007, *A. R. Burgaz*, MACB 96101, MACB 95332; Ojos-Albos, 5-XI-2005, *A. R. Burgaz*, MACB 93603; Peguerinos, campamento de Peñas Blancas, 8-V-2005, *A. R. Burgaz*, MACB 95257, MACB 100477, MACB 100481; Peregrinos, campamento de Peñas Blancas, 8-V-2005, *A. R. Burgaz*, MACB 95829; Piedrahita, 21-XI-2004, *A. R. Burgaz*, MACB 95156; Piedrahita, Puerto de Peñas Negras, 21-XI-2004, *A. R. Burgaz*, MACB 95153; Puerto de Villatoro, 21-XI-2004, *A. R. Burgaz*, MACB 95152; Ramacastañas, 14-XI-2004, *A. R. Burgaz*, MACB 95154; Barcelona: Montseny, P. Nat. del Montseny, 16-VIII-2006, *A. R. Burgaz*, MACB 100476; Burgos: Río de Lunada, montes de Valmera, subida al portillo de Lunada, 25-VII-2006, *A. R. Burgaz*, MACB 93792, MACB 93797; Santa Cruz de Valle Urbión, 30-VII-1996, *N. Marcos & P. Navarro*, MACB 96473; Santa Cruz de Valle Urbión, Sª de la Demanda, 2-IX-1991, *I. Martínez*, MACB 45705; Urrez, Sª de Mencilla, 12-III-2008, *A. R. Burgaz*, MACB 97274; Villasur de Herreros, Sª de San Millán, valle del río Arlanzón, 13-III-2008, *A. R. Burgaz*, MACB 97275; Cáceres: Casas de Miravete, Puerto de Miravete, 9-I-2004, *A. R. Burgaz*, MACB 96240; Guadalupe, Sª de Guadalupe, subida al Puerto Villuercas, 4-V-2007, *A. R. Burgaz*, MACB 94838; Hoyos, Sª de Santa Olalla, 6-IV-1996, *G. Aragón, A. Herrero & I. Martínez*, MACB 95099; La Calera, Sª de la Palomera, 5-V-2007, *A. R. Burgaz*, MACB 94839; Las Mestas, Sª de las Mestas, valle del río Batuecas, 6-XI-2005, *A. R. Burgaz*, MACB 93604; San Martín de Trevejo, Sª de Gata, vertiente sur, 26-VII-1997, *A. R. Burgaz*, MACB 93838; Villamiel, Sª de Gata, aroyo de los Legares, 2-IV-1996, *G. Aragón, A. Herrero & I. Martínez*, MACB 95100; Villareal de San Carlos, Parque de Monfragüe, Sª de las Corchuelas, 5-IV-1991, *F. Sarrión*, MACB 45704; Villareal de San Carlos, P. Nat. de la Sª de Monfrägue, umbría de Monfragüe, 4-V-2007, *A. R. Burgaz*, MACB 95091; Cádiz: Sª del Aljibe, 4-IV-1991, *F. Sarrión*, MACB 45710; Cantabria: Campoó de Cabuérniga, río Saja, 1-IV-1994, *G. Aragón & I. Martínez*, MACB 97210; Calameño, Invernales de Mato, 8-VI-1994, *A. R. Burgaz*, MACB 95107; Camaleño, Fuentede, 15-XI-2003, *G. Amo, A. R. Burgaz, I. Martínez & M. Otalora*, MACB 95749; Camaleño, Invernales de Mato, 8-VI-1994, *A. R. Burgaz*, MACB 64886; Camaleño, Pido, 15-XI-2003, *G. Amo, A. R.*

Burgaz, I. Martínez & M. Otalora, MACB 89758; Ozcaba, el Abrevadero, 5-VIII-1995, *E. Ortega & P. Navarro*, MACB 64894; Vega de Liébana, Tudes, 6-VI-1994, *A. R. Burgaz*, MACB 64891; Ciudad Real: Puebla de Don Rodrigo, Riofrío, 16-VI-1996, *F. J. Sarrión*, MACB 96474; Solana del Pino, Sª de la Solana del Pino carretera vieja hacia Solana del Pino, 4-II-1997, *A. R. Burgaz, I. Martínez & F. J. Sarrión*, MACB 96511; Gerona: L'Estartí, 4-II-1998, *A. R. Burgaz*, MACB 96675; Setcases, estación de esquí Valter 2000, 17-VIII-2006, *A. R. Burgaz*, MACB 95831; Supermolina, Sª del Cadí, 2-VII-1996, *A. R. Burgaz & I. Martínez*, MACB 95101; Guadalajara: Bustares, Sª de Alto Rey, 23-VIII-2003, *A. R. Burgaz*, MACB 95298; Cantalojas, P. Nat. Tejera Negra, 23-VIII-2003, *A. R. Burgaz*, MACB 95289; Cantalojas, barranco río Lillas, P. Nat. Tejera Negra, 23-VIII-2003, *A. R. Burgaz*, MACB 95290; Cardoso de la Sª, Sª de Ayllón, 8-III-1992, *A. Herrero*, MACB 45703; El Cardoso de la Sª, Sistema Central, 20-X-2003, *G. Amo de Paz*, MACB 96533; Gascueña de Bornoba, Sª de Alto Rey, 23-VIII-2003, *A. R. Burgaz*, MACB 95297; Hiendelaencina, 22-IV-2007, *A. R. Burgaz*, MACB 94815; Huesca: Astún, valle de Canfranc, 28-VII-2000, *A. R. Burgaz*, MACB 96718; Ibón de Baños, 5-IX-1991, *T. Ahti & A. R. Burgaz*, MACB 45709; Panticosa, subida al Ibón de Brazato, 7-XI-1994, *A. R. Burgaz, I. Martínez & F. J. Sarrión*, MACB 96512; Plan, refugio de Labasar, valle de Gistau, 27-VII-2003, *A. R. Burgaz*, MACB 97665; Saravillo, hacia refugio Labasar, valle de Gistau, 27-VII-2003, *A. R. Burgaz*, MACB 95292; La Coruña: Betazos, Espenuca, fraga de Chelo, 22-X-1994, *A. R. Burgaz & I. Martínez*, MACB 64890; La Rioja: Anguiano, valle del río Najerilla, 8-IX-2004, *A. R. Burgaz*, MACB 89738; Lumbreras, 20-X-1983, *A. R. Burgaz & Mendiola*, MACB 14663; Lumbreras, 21-X-1983, *A. R. Burgaz & Mendiola*, MACB 14652; Mansilla de la Sª, 8-IX-2004, *A. R. Burgaz*, MACB 89525; Mansilla, barranco de Mansilla, 19-IX-1990, *A. R. Burgaz*, MACB 45697; Montenegro de Cameros, 5-VI-2003, *A. R. Burgaz*, MACB 97666; Sª de la Hez, 21-IX-1990, *A. R. Burgaz*, MACB 45696; Valdezcaray, Sª de la Demanda, 30-X-2002, *A. R. Burgaz*, MACB 96592; Venta de Piqueras, 21-X-1983, *A. R. Burgaz & Mendiola*, MACB 37093; Villoslada de Cameros, Sª de Cebollera, 8-XII-2007, *A. R. Burgaz*, MACB 96350; León: Colinas del Campo de Martín Moro, 21-X-1994, *A. R. Burgaz & I. Martínez*, MACB 64893, MACB 96475; Manzanal del Puerto, 3-IX-2002, *A. R. Burgaz*, MACB 96233; Puerto de las Señales, 6-VIII-2003, *A. R. Burgaz*, MACB 95291; Puerto de San Isidro, 5-VIII-2003, *A. R. Burgaz*, MACB 96241; Salientes, Palacios del Sil, 28-III-2002, *R. Garác*, MACB 95157; Tejedo de Ancares, 11-VII-1984, *A. R. Burgaz*, MACB 45664; Lérida: Ars, Valls de Vallira, 1-VII-1996, *A. R. Burgaz & I. Martínez*, MACB 96234; Artiga de lin, valle de Arán, 26-VII-1998, *A. R. Burgaz*, MACB 95103; Bosost, valle de Arán, 21-VIII-1993, *I. Martínez*, MACB 64880; Espot, pista de Lladres, Riu de Peguera, 12-VII-1994, *G. Aragón, J. Castillo, A. Herrero & I. Martínez*, MACB 64881; Espot, subida lago de San Mauricio, 27-VII-1998, *A. R. Burgaz*, MACB 95102; Espot, vall d'Espot, 13-VII-1994, *G. Aragón, J. Castillo, A. Herrero & I. Martínez*, MACB 96593; Lugo: Cubelas, Castroverde, 2-I-1993, *E. Munín*, MACB 96154; Gundriz, Municipio Samos, valle de Louzara, 24-III-2005, *A. Noguerol Seoane*, MACB 95748; Madrid: El Berrueco, 9-XI-2007, *A. R. Burgaz*, MACB 95985; La Acebeda, Sª de SomoSª, camino del puerto de la Acebeda, 14-I-2007, *A. R. Burgaz*, MACB 100476; Lozoya, Puerto de Navafría, 15-X-2003, *A. R. Burgaz*, MACB 96516; Miraflores de la Sª, 27-X-2006, *A. R. Burgaz*, MACB 95178; Miraflores de la Sª, puerto de Canencia, 14-II-2000, *A. R. Burgaz & I. Martinez*, MACB 96239; Miraflores de la Sª, subida al puerto de Canencia, 8-IV-1996, *A. R. Burgaz*, MACB 95105; Montejo de la Sª, 8-III-2002, *P. Aguilar, G. Amo de Paz & A. R. Burgaz*, MACB 95104; Navalagamella, 17-I-1992, *A. Herrero*, MACB 45707; Puerto de Canencia, 13-XI-1998, *A. R. Burgaz*, MACB 70265; Puerto de Canencia, 29-X-1990, *A. R. Burgaz*, MACB 45700; SomoSª, Puerto de SomoSª, arroyo de la Peña del Chorro, 9-XI-2007, *A. R. Burgaz*, MACB 95984; Navarra: El Zaburu, 9-IX-1991, *T. Ahti & A. R. Burgaz*, MACB 45701; Oronoz-Mugaire, Valle del Baztan, 8-IX-1991, *T. Ahti & A. R. Burgaz*, MACB 45702; Orense: Subida a Cabeza de Manzaneda, Manzaneda, Sª de Queixa, 24-VIII-2002, *A. R. Burgaz*, MACB 96238; Subida a Cabeza de Manzaneda, Sª de Queixa, 24-VIII-2002, *A. R. Burgaz*, MACB 96235; Palencia: Cardaño de Arriba, Sª de Alba, 18-VII-2004, *A. R. Burgaz*, MACB 95158; Cardaño de Arriba, Sª de Alba, 18-VII-2004, *A. R. Burgaz*, MACB 95159; Vidrieros, Reserva Nacional de Fuentes Carrionas, Alto de Riofrío, 23-VIII-2006, *A. R. Burgaz*, MACB 93796; Salamanca: Beleña, 5-XI-2005, *A. R. Burgaz*, MACB 93605; Carpio de Azaba, 1-V-2007, *A. R. Burgaz*, MACB 95255; El Cabaco, Sª de la Peña de Francia, 6-XI-2005, *A. R. Burgaz*, MACB 93606; El Casarito, Sª de la Peña de Francia, 6-XI-2005, *A. R. Burgaz*, MACB 93607; Fuenterroble de Salvatierra, Sª de Frades, 5-XI-2005, *A. R. Burgaz*, MACB 93608; La Alberca, Sª de las Mestas, valle del río Batuecas, 6-XI-2005, *A. R. Burgaz*, MACB 93611; Peña de Francia, 26-IX-1991, *A. R. Burgaz*, MACB 45694; Valle del Regato Rubioso, 5-IV-1996, *F. J. Sarión*, MACB 95106; Segovia: Aguilafuente, 14-XI-1993, *A. R. Burgaz*, MACB 96476; Aldehuela de Pedraza, subida al Puerto de Navafría, 26-I-2008, *A. R. Burgaz*, MACB 96755; Coca, Finca El Sequero, 20-VII-2006, *A. R. Burgaz*, MACB 93794; El Espinar, valle del río Moros, área recreativa la Panera, 26-I-2008, *A. R. Burgaz*, MACB 96752; La Granja, Valsaín, río Eresma, fuente de los Dos Caños, 26-I-2008, *A. R. Burgaz*, MACB 96754; Revenga, cordel de Peñas Zamarriegas, 26-I-2008, *A. R. Burgaz*, MACB 96753; Riofrio de Riaza, Puerto de la Quesera, 29-VI-1991, *A. R. Burgaz*, MACB 45695; Soria: Almazán, 6-IV-1993, *A. R. Burgaz*, MACB 96477; Laguna Negra, 7-IX-2003, *A. R. Burgaz*, MACB 93840; Lubia, Altos de Lubia, 7-IX-2003, *A. R. Burgaz*, MACB 95293; Teruel: Orihuela del Tremedal, Sª de Albarracín, arroyo Gargantavellanos, 19-V-1996, *A. R. Burgaz*, MACB 96155; Puerto de Orihuela del Tremedal, 11-VI-1991, *A. R. Burgaz*, MACB 45699; Toledo: Hontanar, Montes de Toledo, 5-I-1995, *G. Aragón & I. Martínez*, MACB 96517; La Iglesuela, Sª de San Vicente, 17-II-2002, *A. R. Burgaz & I. Martínez*, MACB 95108; Los Navalucillos, Montes de Toledo, arroyo del Chorro, 11-II-1995, *G. Aragón, A. Herrero & I. Martínez*, MACB 96478; Navamorcuende, ascenso a las Cruces vert S, 23-III-1996, *S. Vázquez, A. R. Burgaz & M. Acón*, MACB 60295; Navas de Estena, 8-III-1991, *I. Martínez*, MACB 45706; Valladolid: Pedrajas de San Esteban, 20-VII-2006, *A. R. Burgaz*, MACB 93793; Zamora: La Tabla, 6-X-1997, *G. Aragón, A. R. Burgaz & A. Terrón*, MACB 70224; Porto, Laguna Sanabresa, P. Nat. "Lago de Sanabria", 9-IX-1998, *A. R. Burgaz, S. Casas & I. Rodríguez de Lope*, MACB 70225; Ribadelago, subida Pico del Fraile, P. Nat. "Lago de Sanabria", 9-IX-1998, *A. R. Burgaz, S. Casas & I. Rodríguez de Lope*, MACB 70217; Torregamones, Arribes del Duero, 12-VIII-2001, *A. R. Burgaz*, MACB 95109; Zaragoza: Circo de San Miguel, Sª del Moncayo, 13-IX-2003, *A. R. Burgaz*, MACB 95294; Tarazona, Barranco de Castilla, 4-IX-1984, *A. R. Burgaz*, MACB 45698; Vera de Moncayo, 13-IX-2003, *A. R. Burgaz*, MACB 100474; Vera de Moncayo, Sª del Moncayo, 13-IX-2003, *A. R. Burgaz*, MACB 93839; Canary Islands: El Hierro, Frontera, P. Nat., 5-X-2001, *N. Marcos & D. Manso*, MACB 97667; La Palma, Barlovento, 26-XI-1993, *A. R. Burgaz*, MACB 96673; Tenerife, La Esperanza, Monte de la Esperanza, corona forestal, 16-VI-2007, *A. R. Burgaz*, MACB 97933; Tenerife, La Esperanza, Monte de la Esperanza, pista del Acebiño, 16-VI-2007, *A. R. Burgaz*, MACB 97932; Tenerife, La Orotava, subida al Teide, 15-VI-2007, *A. R. Burgaz*, MACB 97931; **Sweden**: Dalarna: Transtrand par, Hamrarna, 0,5 Km ONO Gåstjärnen, 10,8 Km NO Transtrand k:a, 20-VII-1989, *J. Hermansson & L. E. Muhr*, UPS L-175253; Halland: Älvkarñeby par., Varås, 6-V-1919, *C. Stenholm*, UPS L-144725; Hälsingland: Mo par., Between Mohed Airport abd Road 301, 21-IX-2003, *A. Nordin*, UPS L-132020; Sverige: Gästrikland, Torsaker sn, 850 m. SO om Ältebo, det sydligaste huset, 15-VII-2005, *G. Odelvik 05946*, S F52879; Hälsingland, Ramsjö sn, 2000 m. N-NNO om Ramsjö kyrka, 24-VII-2007, *G. Odelvik 07545*, S F90966; **Switzerland**: Zug: Heilogkreuz, 4-VII-1954, *W. B.*, L 794556; **United Kingdom**: Scotland: Corriemoillie, Westerm Ross, Higland Dist, 12-VI-2002, *A. R. Burgaz*, MACB 97930; Glendrian, 25-VII-2004, *L. Spier*, L 752939; **United States**: South Dakota: Lawrence, 3 miles southwest of central city along Deadwood Creek (2,5 miles west of Lead). Along nort facing slope up from stream, 23-VIII-1960, *C. M. Wetmore*, BRACR 10052; **Unknown**: BRACR 10042;

Schlankere Lagerstiele, einfach, spitzing aber etwas adgestutzi, teils mehr oder weniger verätels, X-1927, *J. Anders*, BRACR 10055.

***Cladonia symphycarpa* (Flörke) Fr. Sched.**

Andorra: Ordino, coll de Ordino, 19-VIII-2006, *A. R. Burgaz*, MACB 93500; **Argentina**: Santa Cruz: Piedra Blance Glaciar, I-2007, *I. Garibotti*, H; Tierra de Fuego: Isla Grande, río Grande, 3 Km E of Estancia Despedida, 6-I-1989, *T. Ahti*, H; **Austria**: Kärnten Gösseringbach; Weibbriach, 19-VII-1994, *L. Spier*, L 0752979; Steiermark: Nördliche Kalkalpen, Ennstaler Alpen, Gesäuseberge, NE above the Oberst-Klinke-Hütte, path from Kalblinggatterl to the Kalbling, S below the steep rocjs, 26-V-2000, *J. Hafellner*, UPS L-135579; Nördliche Kalkalpen, Ennstaler Alpen, Gesäuseberge, NE above the Oberst-Klinke-Hütte, path from Kalblinggatterl to the Kalbling, S below the steep rocjs, 26-V-2000, *J. Hafellner*, CANB 774605; Styria: Eisenezer Alpen, 6 Km N of Kalwang, right of the Achner Alm, 11-V-1997, *J. Miadlikowska, A. Hafellner & J. Hafellner*, H; **Bosnia Herzegovina**: Sarajevo: Trevici, 28-III-2010, *A. R. Burgaz*, MACB 101124; **Canada**: Brithish Columbia: Creek Research area, 10 Km ESE of Sicamous, 7-VIII-1993, *T. Goward*, H; Haines Triangle, 18 Km of confluence of Tatshenshini and Alsek, 22-VII-1992, *T. Goward, W.B. Schofield & G. Godfrey*, H; Liard river basin (Northern Rocky Mountains), Wokkpash Lake, 21-VII-1977, *I. M. Brodo & P. Hamilton*, H; Sicamous Creek Research area, 10 Km ESE of Sicamous, 8-VIII-1993, *T. Goward*, H; Vancouver Island, Goldstream, Mt. Wells, 30-IX-1958, *T. Ahti*, H; Manitoba: SE of Flin Flon, Little Spruce Lake Road (off North Star Road), 24-V-2004, *T. Ahti, M. Piercey-Normore & T. Booth*, H; Newdoundland: Labrador, Menihek Lake, 9-VII-1966, *I.M. Brodo*, H; Ontario: Ottawa-Carleton County, 15 Km S of Richmond, 15 Km W of Kemptville, 26-IX-1985, *I. M. Brodo*, H; Yukon territory: Big Creek Camp Ground mile 674 Alaska Hwy. 40 mile W of Watson Lake, 13-VII-1967, *T. Ahti*, H; Kluane Lake, mile 1064 Alaska Hwy, 21-VII-1967, *T. Ahti*, H; Watson Lake town, by Centennial Avenue, 4-VII-1977, *T. Ahti & G. W. Scotter*, H; **Cuba**: Oriente: Roca de la Gran Piedra, Sª Maestra, 16-VIII-1959, *H. A. Imshaug*, H; **Finland**: Åland Islands: Eckerö, Norrsundetin SE-puolella, 20-VI-1986, *M. Kuusinen*, H; Eckerö, Signilskä, 15-VI-1969, *C. A. Haeggström*, H; Eckerö, Storby, 1-VII-1954, *J. Suominen*, H; Eckerö, Storby, Sandviken, 30-VI-1954, *J. Suominen*, H; Eckerö, Torpin eteläosa, SW-niemestä 1 km NE, 19-VII-1962, *T. Koponen*, H; Jomala, Önningby, Hällmark ca. 500 m w on Lystarklint, 29-VIII-1982, *C. A. Haeggström & R. Skytén*, H; Lemland Natö, Munkolmen, Hällmark, 30-VII-1981, *C. A. Haeggström & R. Skýten*, H; Lemland, Lemböte, Granholm, 31-VIII-1983, *C. A. Haeggström & R. Skytén*, H; Lemland, Norrby, Segelgrundet Hällmark, 10-VII-1969, *C. A. Haeggström*, H; Finland Proper: Archipelago of Turku, Velkua, Niitly-Saukkoluoto island, 3-VIII-1971, *R. Alava & S. Hinneri*, H; Houtskär, Hyppeis, 25-VIII-1995, *H. Bruun*, H; Karjalohja, Pellonkylä, 18-VII-1882, *J. P. Norrlin*, H; Korpo, Avensor, Kälklot, 5-VII-1988, *R. Skytén*, H; Korpo, Avensor, Kirmo, 5-VII-1988, *R. Skytén*, H; Korpo, Jurno, 4-VII-1988, *R. Skytén*, H; Lohjan Kunta, Paloniemi, Rauhalahdesta NW, 18-IX-1960, *M. J. Kotilainen*, H; Parainen, west side of Ravalangsbergen hill, 15-V-1973, *S. Hinneri & U. Laine*, H; Särkisalo, Förby village, 10-VI-1964, *R. Alava, K. Alho, U. Laine*, H; Korpo, Avensor, islet Kälkot, 5-VII-1988, *O. Vitikainen*, H; Korpo, island Jurmo in outer archipelago, 23-VII-1985, *T. Ahti*, H; Korpoo, Jurmo, SE of Moringharu, 23-VIII-1985, *I. Kytövuori*, H; Lohja, Isoteutari, 21-IX-1957, *T. Ahti*, H; Northern Ostrobothnia: Kuusamo Paarajärvi Ruskiakallio, 9-VII-1934, *Räsänen*, L 794534; Uusimaa: Bromarf, Bergö, N om Solböle, 24-VII-1945, *H. Buch*, H; Finby, Mustajuuri, 1920, *F. Elfving*, H; Karislojo, Pellonkylä, pa kalkhällar, 14-VIII-1890, *C. E. Boldt*, H; Karjalohja, 10-IX-1990, *J. Pykälä*, H; Karjalohja, Pitkälahti, Kellinki, NW-reunan, 14-VIII-1990, *J. Pykälä*, H; Karjalohja, Pyöli, Nikuska runsaat 200 m S-SE, 20-VIII-1996, *J. Pykälä*, H; Karjalohja, Saarenpää, Alminmäestä, 13-VI-1986, *J. Pykälä*, H; Karjalohja, Saarenpää, Saarenpäänniemi, NW-kärki, 14-X-1991, *J. Pykälä*, H; Karkkila, Haavisto, Koirakallio, 31-V-1992, *J. Pykälä*, H; Lohjan kunta, Torhola, Karkalinniemen S-ranta, 23-VIII-1990, *J. Pykälä*, H; Lojo, Karhuniemi, kalkhaltigt, 26-VIII-1946, *E. F. Hällström*, H; Lojo, vid Lojobacke kalklmoll, 5-X-1892, *C. E. Boldt*, H; Suomusjärvi, Kitula, Kirjun vanha kalkkilouhos, 10-V-1988, *M. Heino*, H; Västanfjärd, Illogruvan, 9-VI-2005, *V. Haikonen*, H; Västanfjärd, Västerillo, 11-V-1961, *J. Suominen*, H; **Germany**: Angsburg: Leehfeld, *M. Britzelmayr*, H; Baden-Württemberg: Kreis Göppingen, Schwäbiche Alb bei Winzingen am Heldenberg, 14-VIII-1970, *Schumm*, H; Brandenburg: Kr. Märkisches Oderland Odertalradhänge SO des Ortes Libbenichen, Grenzberg und Hänge südlich davon, 1-V-2003, *H. Sipman & S. Rätzel*, B 60 0122320; North Rhine-Westphalia: Kr. Schleoden, Nettersheim, Mannenberg, Trig. Punkt, Mesobrometum, 20-IX-1964, *H. Breuer*, B 60 0146524; Kr. Schleoden, Nettersheim, Mannenberg, Trig. Punkt, 20-IX-1964, *H. Breuer*, B 60 0143645; Sachsen-Anhalt: Auf Muschelkalk bei Köllme, 31-V-1970, *S. Huneck*, B 60 0125304; Schmoner Bruch, Spielberg, Spielberg Höhe, 7-VIII-1994, *S. Huneck*, B 60 0125267; Thuringia: Alter Stolberg bei Nordhaufen am Harz auf Gypsboden, 1920, *Sandstede*, H; Alter Stolberg, bei Nordhausen am Harz., X-1920, *Sandstede und Wein*, UPS L-104192; **Iceland**: Central Higlands, S of Hofsjökull Glacier, Hnífárver, 27-VII-1970, *H. Kristinsson*, H; **Iran**: Mazandaram: Nour, Kojdur and Kodir villagies, 3-IV-2002, *M. Sohrabi & M. Mofid*, H; **Mongolia**: East Hangay: Mts. Arhangay aimak, somon Tövshruuleh, 10-VIII-1970, *L. G. Biazrov*, H; Mts. Arhangay aimak, somon Tövshruuleh, 20-IX-1979, *L. G. Biazrov*, H; **North Korea**: Gangwon-do: Kumgagsan, Onjong-ri, auf Felsblock, 29-IX-1988, *S. Huneck*, H; **Norway**: Nordland: Rana, Dunderlandsdalen, E bank of river Messingaga, junt S of E6/Ranaelva, 6-VIII-2006, *T. Tonsberg*, BG L84035; **Poland**: Lesser Poland: Pieniny Czorstynskie (pow. Nowy Targ.) Na ziemi przy drodze pomiedzy Potokiem Kirowym a Potokiem Czarnym. Carpati Occ., 13-IX-1957, *Z. Tobolewski*, L 794533; **Russia**: Karelia Republic: Kb, Suojärvi, Varpakylä, Pöpönsaari, 19-VII-1914, *K. Linkola*, H; Par. Suojärvi, 1870, *J. P. Norrlin*, H; Krasnoyarsk: North of Central Siberia, Taimyr Peninsula, near Belyi Yar at the north-western coast of Pyasino Lake in the vicinities of Nyapan hill, 27-VII-1999, *L. Zanokha*, H; Taimyr National area by mouth of Pyasino River, vicinity of Lake Pyasino, 17-VII-1983, *M. P. Zhurbenko*, H; Taimyr National area, Taimyr Peninsula, S coast of the Kara sea, region of the mouth of Uboynaya River, 3-VIII-1990, *M. P. Zhurbenko*, H; Taimyr National area, Taimyr Peninsula, S coast of the Kara sea, region of the mouth of Uboynaya River, 3-VIII-1990, *M. P. Zhurbenko*, H; Magadan Oblast: Center of Vragel Island near headwaters of the Neizvestnaya River, 26-VII-1987, *S. Kholod*, H; Sakha Republic: Kangalassy Dist., National Nature Park Lenskie Stolby, south (rigth of Labyiya River, 30-VI-2002, *T. Ahti*, H; Tuva Republic: South Siberia Mountains, Todginskaya Valley, State Reserve "Azas", Azas River in its lower part, 24-VIII-1997, *T. N. Otnykova*, H; **Spain**: Álava: Laguardia, subida a la Cruz del Castillo, 10-XI-2007, *R. Pino-Bodas*, MACB 192687; Leza, Sª de Cantabria, subida al Pto de Herrera, 26-VII-2006, *A. R. Burgaz*, MACB 93499; Peña Cerrada, Sª de Cantabria, Puerto de Herrera, 26-VII-2006, *A. R. Burgaz*, MACB 93502; Tertanga, Puerto de Orduña, 25-VII-2006, *A. R. Burgaz*, MACB 93501; Ávila: Ojos-Albos, 5-XI-2005, *A. R. Burgaz*, MACB 102701; Burgos: Campino, 19-VIII-2008, *A. R. Burgaz*, MACB 102705; Escalada, páramo, 19-VIII-2008, *A. R. Burgaz,* MACB 102686; Vivanco de Mena, Valle de Mena, 25-VII-2006, *A. R. Burgaz*, MACB 102682, MACB 93496; Cantabria: Entrambasaguas, Sª de Peñalabra, río Hijar, 241/1/1970VII-2006, *A. R. Burgaz*, MACB 93503; Cuenca: Vadillos, hoz de Beteta, fuente de los Tilos, valle del río Guadiela, Serranía de Cuenca, 16-V-2008, *A. R. Burgaz*, MACB 102682; Gerona: El Volcan Santa Margarita, 22-IV-1977, *F. Adema & F. Aleva*, L 794532; San Martín de Ogassa, mirador coll de la Torre, 17-VIII-2006, *A. R. Burgaz*, MACB 102694, MACB 93504, MACB 93504; Guadalajara: Gascueña de Bornoba, Sª de Alto Rey, 23-VIII-2008, *A. R. Burgaz*, MACB 93558; Jadraque, 14-IV-2006, *R. Pino-Bodas*, MACB 102704; Sigüenza, 12-X-2002, *A. R. Burgaz*, MACB 93559; Huesca: Bielsa, valle de Pineta, P. N. de Ordesa y Monte Perdido, 28-VII-1998, *A. R.*

Burgaz, MACB 92738; Bielsa, valle de Pineta, río Cinca, 25-VII-2008, *A. R. Burgaz*, MACB 102697; Gistain, valle de Gistau, 27-VII-2003, *A. R. Burgaz*, MACB 102700; Santa Cruz de la Serós, Sª de San Juan de la Peña, monasterio alto de S. Juan de la Peña, 21-VIII-2006, *A. R. Burgaz*, MACB 102699; Santa María de Buil, 25-VII-2008, *A. R. Burgaz*, MACB 102698; La Rioja: Anguiano, valle del río Najerilla, 8-IX-2004, *A. R. Burgaz*, MACB 102690; León: Isoba, eserva Nac. de Mampodre, Pto. de S. Isidro, laguna de Isoba, 22-VII-2006, *A. R. Burgaz*, MACB 102696; Lérida: La Coma i la Pedra, Sª del Port del Compte, camino de la estación de esqui, 4-VII-1996, *A. R. Burgaz & I. Martínez*, MACB 92778; La Vansa-Fornols, Ges, Sª del Cadí, 3-VII-1996, *A. R. Burgaz & I. Martínez*, MACB 92740; Pas de la Casa, Coll de la Bonaigua, 20-VIII-2006, *A. R. Burgaz*, MACB 102692; Madrid: Redueña, 11-V-1998, *A. R. Burgaz*, MACB 92737; Navarra: Isaba, Piedra de San Martín, 27-VII-2008, *A. R. Burgaz*, MACB 102695; Sª de Urbasa, 27-IX-1999, *A. R. Burgaz & Rodrígues de Lope*, MACB 92780; Palencia: Piedrasluengas, desfiladero del arroyo Lazán, 20-VIII-2008, *A. R. Burgaz*, MACB 102703; Puerto de Piedrasluengas, 29-IX-1999, *A. R. Burgaz & Rodrígues de Lopez*, MACB 92739; Soria: Muriel Viejo, 12-VIII-2008, *A. R. Burgaz*, MACB 102689; Ucero, cañón del río Lobos, ermita de San Bartolomé, 25-X-2008, *A. R. Burgaz*, MACB 102689; Villaciervos, Altos de Villaciervos, 10-IX-1991, *T. Ahti & A. R. Burgaz*, H; Teruel: Fonfría, Sª del Cucalón, 23-VI-1992, *A. R. Burgaz*, MACB 102702; Villar del Cobo, *A. R. Burgaz*, MACB 102844; Valencia: Utiel, Casas de Medina, Sª del Negrete, Alto de Negrete, 28-II-2009, *A. R. Burgaz*, MACB 102691; **Sweden**: Gotland: Eksta par., Stora Karlsö, Röjsu heid, 26-VI-1969, *G. Degelius*, UPS L-75598; Othem par., Söjdbro. Alvarmark, 30-VIII-1949, *G. Degelius*, UPS L-75600; Öland: Resmo sn, 5000 m OSO om Resmo kyrka, 28-VIII-2002, *Göran Odelvik*, S L50055; **Switzerland**: Ct. Vaud. Col du Pillon, 0.5 km E of hotel, 28-V-1969, *T. Ahti*, H; **Turkey**: Trabzon: 50 Km S of Trabzon, Meryemana forest, 1990, *G. Cevahir*, H; **Ukraine**: Donests'k Oblast: Shakhtarsky District, steppe slops near Petrivs'ke village and Sevostianovka River, Regional Landscape Park "Donetsk upland", 17-IV-2006, *O. Nadeina*, H; Luhansk Oblast: Antracit Distric, to the NW from Malomykolaivka village, 6-V-2005, *O. Nadeina*, H; Lutugynsky District, to the N from Verkhnia Orikhivka village, 4-V-2005, *O. Nadeina*, H; **United States**: South Carolina, *H. W. Ravenel*, L 794536; Alaska: Crow Pass, Turnagain Arm, 9-VIII-1993, *C. Derr*, H; Kansas: Greenwood county, ca. 11 milles NW of Fredonia, 24-VIII-1960, *R. A. Anderson & S. Shushan S-29, 124*, FR 59999; Massachessetts: Berkshire County, town of Cheshire, 400 m south of Curran Rd., 5-V-1995, *M. S. Cole*, H; Michigan: Delta County, in peninsula dicta "Garden" ad meridiem a Portage Bay Campground, 15-IX-1976, *R. C. Harris*, H; Mackinac County, Hiawatha National Forest, 2 miles SW of East Lake (11 mi SE of Trout Lake), 15-VII-2004, *C.M. Wetmore No. 91877*, S F53075; Vheboygan Co., Douglas Lake, c. 0.5 Km S of University of Michigan Biological Station, 19-VIII-1977, *T. Ahti*, H; Montana: Missoula County, near Morrell Fall, Swan Range, 20-VII-1984, *B. McCune*, H; Oregon: Jackson Co., Table rock (NW of Medford), Lower Table Rock, 13-VI-1984, *T. Ahti & D. H. Norris*, H; **Uruguay**: Maldonado: Sª de Las Ánimas, Las Flores (95 Km E of Montevideo on Highway 9), 25-I-1989, *S. Stenroos, T. Ahti & H. S. Osorio*, H; **Unknown**: 1995, *K. Baimam*, FR 50141; 9-V-1954, *H. Stadler 2035*, FR 51908; Hirschegg, Schwarzwassertal; zwischen Auenhütte und Melköde bei Reuhe, 21-VIII-1967, *Klement*, FR 75495; N. Lgalië, Otzlaten, O. van Giaum, Langtaufesen Tal, VI-1906, *R. Hengeveld*, L 794535; S.S.R. Grow Woronesh Dorh Gulorew, VII-1926, *Samin*, H; Schobersheim im Höllengelinge, 23-III-1967, *F. Grims 7314*, FR 75494; Vogtland, an sonnigen Hügeln bei Pirk an der Elste, VII-1924, *E. Stolle & Dr. Schade 18271*, FR 75497.

***Cladonia thomsonii* Ahti**

Canada: Newfoundland: Mackenzie District, Crossley Lakes, 12-VI-1966, *G. W. Scotter*, H; **Russia**: Krasnoyarsk: N of Central Siberia, Severmaya Zemlya archipelago, N part of Bol'shevik Is., W coast of Akhmatov Bay, 7 Km SW of the Bazavaya, 15-VII-1996, *M. Zhurbenko*, H; Magadan Oblast: Wrangell Island, 5-VIII-1933, *A. U. Mineev*, H; **United States**: Alaska: Chugach Mts., Thompson Pass mile 25.2 Richardson Hwy., 27-VII-1967, *T. Ahti & J. W. Thomson*, H; Alaska: On the point of Point Barrow, 18-VII-1958, *J. W. Thomson, S. Shushan & A. J. Sharp*, H.

***Cladonia turgida* Hoffm.**

Canada: Newfoundland: Harbour Main Dist., Brigus, SE margin of the village, 15-IV-1999, *T. Ahti*, H; Trout River, behind Crocker Cabin, 18-VII-2006, *C. Lendemer et al.*, H; Ontario: Bruce county, Bruce Peninsula National Park, NW of Emmett Lake Road, 2.7 Km NE of Hwy 6, 21-IX-2008, *J. C. Lendemer*, H; Hwy. Q7 nr. White Lake, 9-VIII-1988, *S. Hammer*, H; **Greenland**: Nanortalik, 6-VII-1993, *E. S. Hansen*, H; Narsaq, Kvanefjeld, 30-VII-1982, *S.Svane*, H; **Iceland**: lau. S-Múlasýsla, Oddskaro between Eskifjörour and Norofjörour, 15-VII-1992, *S. Svane*, H; NW-side, Isafjardhar Sýsla, Seljalandsdalur, 28-VIII-1966, *P. Oosterveld*, H; **Norway**: Østlandet: Akershus, Frogn hd, Söndre Hallangen, Marikova, 5-VI-1960, *T. Ahti*, H; Østlandet: Buskerud, Kongsbergs kn Hedenstad, Rajehogda SW, 24-VIII-1982, *G. Krist*, H; **Russia**: Kaliningrad Oblast: Oftpreufen, Rominter Heide, Kreis Goldap, 10-IX-1922, *Führer*, H; Moskva Oblast: Prioksko-Terrasny Zapovednik, on the terraces of the Oka river, 12 Km E of Serpukhov, ca. 30 Km S of Moscu, 5-VIII-1979, *H. H. Iltis, J. C. Coffey, M. F. Denton & V. I. Danilov*, H; Sakha Republic: Siberia, Yakutia, New Siberian Islands, Bennett Island, 1-VIII-1989, *M. P. Zhurbenko*, H; Sakhalin Oblast: Kuril Is., Far esat Paramushir Island, 4-VIII-1988, *Koroleva*, H; **Sweden**: Götaland: Västergötland, V. Tunhem, 18-VII-1899, *C. Stenholm*, H; Torne Lappmark: Jukkasjärvi, Talvimaa, Maltosuvanto, 3-IX-1911, *G. Lang*, H; Karesuando, Taavaskaite, Laukkuoivi, 22-VIII-1910, *M. Montell*, H; Uppland: Erhart, Upsaliae, H.

ANEXO 2: Material suplementario del ARTÍCULO IX

Table 1S. List of specimens used in this study with GenBank accession numbers, new sequences in bold. - = PCR failed, ? = no tested.

Species	Sample	Country	Date	Voucher	ITS rDNA	*rpb2*	IGS rDNA	*ef1α*	*cox1*
Cladonia acuminata	1ACUMI	USA	25-VI-2004	H	JN621932	JN621965	-	JN621996	?
Cladonia acuminata	2ACUMI	Canada	25-V-2004	H	JN621933	JN621966	-	JN621997	?
Cladonia acuminata	5SYMP	Spain	29-IX-1999	MACB 92739	JN621922	JN621955	-	JN621987	?
Cladonia acuminata	11SYMP	Canada	24-V-2004	H	JN621928	JN621961	-	JN621992	?
Cladonia acuminata	3SYMP	Chile	21-I-2005	MACB 92017	JN621920	JN621953	-	JN621985	?
Cladonia apodocarpa	1APODO	USA	22-IV-2008	H	**+**	**+**	**+**	**+**	?
Cladonia callosa	1CALLO	Denmark	13-IX-1992	H	**+**	-	?	**+**	?
Cladonia cariosa	11CARI	Finland	25-V-2008	H	JN621915	JN621947	?	JN621905	?
Cladonia cariosa	13CARI	Russia	28-IX-2004	H	JN621917	JN621847	?	JN621980	?
Cladonia cariosa	15CARI	Canada	25-V-2004	H	JN621934	JN621950	?	JN621981	?
Cladonia cariosa	4CARI	Spain	13-VI-1991	MACB 45292	JN621908	JN621940	?	JN621972	?
Cladonia cariosa	5CARI	Spain	20-VIII-2006	MACB 94208	JN621909	JN621941	?	JN621973	?
Cladonia cariosa	8CARI	USA	9-VII-2004	S F53032	JN621912	JN621944	?	JN621976	?
Cladonia cariosa	9CARI	Norway	11-VIII-2004	BG L79658	JN621913	JN621945	+	JN621977	?
Cladonia cariosa	12CARI	Finland	9-X-2007	H	JN621916	JN621948	?	JN621979	?
Cladonia cariosa	3CARI	Spain	16-VIII-2006	MACB 94207	JN621907	JN621939	?	JN621971	?
Cladonia cenotea	1CENO	Denmark	14-III-2009	J. Voldrák 6965	FN68596	HM243221	?	HM243199	?
Cladonia cervicornis	13CER	Spain	11-VIII-1996	MACB 91610	FM205916	-	?	?	?
Cladonia cervicornis	1CER	Spain	21-IX-2004	MACB 91631	FM211897	-	?	?	FM208169
Cladonia cervicornis	5CER	Spain	23-VIII-2003	MACB 90738	FM205904	FM2057578	?	?	FM208154
Cladonia cervicornis	7CER	Spain	9-IV-2004	MACB 90840	FM205905	-	?	?	FM208170
Cladonia cervicornis	9CER	Spain	11-V-2004	MACB 90718	FM205906	-	?	?	FM208171
Cladonia coniocraea	8CORN	USA	4-VII-2008	H	JN811384	JN811427	JN811349	JN811436	?
Cladonia coniocraea	13CONI	Sweden	21-VIII-2006	S F60224	**+**	-	**+**	**+**	?
Cladonia coniocraea	19CONI	USA	15-IX-2007	FH	JN811378	JN811336	JN811344	JN811437	?

Species	Sample	Country	Date	Voucher	ITS rDNA	*rpb2*	IGS rDNA	*ef1α*	*cox1*
Cladonia coniocraea	20CONI	Finland	21-VIII-2008	H	JN811379	JN811337	JN811345	-	?
Cladonia coniocraea	21CONI	Russia	3-VII-2004	H	JN811380	JN811338	JN811346	JN811438	?
Cladonia coniocraea	22CONI	Russia	19-VIII-2005	H	JN811381	JN811339	JN811347	JN811439	?
Cladonia coniocraea	24OCHRO126	Unknown	21-IX-2007	H	JN811376	JN811409	JN811342	JN811442	?
Cladonia coniocraea	24OCHRO139	Finland	16-X-2009	MACB 101648	JN811377	JN811410	JN811343	JN811443	?
Cladonia coniocraea	2CONI	Spain	9-XI-2003	MACB 93729	+	+	+	+	?
Cladonia coniocraea	4CONI	Spain	12-VI-2007	MACB 95199	+	+	-	+	?
Cladonia coniocraea	5CONI	Spain	7-IX-2003	MACB 93728	+	+	+	+	?
Cladonia coniocraea	10CONI	USA	6-IX-2003	S L59663	-	+	-	+	?
Cladonia coniocraea	11CONI	Sweden	12-IV-1979	H	-	-	+	+	?
Cladonia coniocraea	11OCHRO	Sweden	22-IX-2001	S L44430	-	-	-	+	?
Cladonia coniocraea	12CONI	Sweden	7-VIII-1960	H	-	-	+	+	?
Cladonia coniocraea	12OCHRO	Germany	X-1918	H	-	-	-	+	?
Cladonia coniocraea	13OCHRO	Sweden	6-X-2003	S L60530	-	-	-	+	?
Cladonia coniocraea	14CONI	Belgium	2-V-1999	UPS L 0753073	-	-	+	-	?
Cladonia coniocraea	14OCHRO	Germany	5-II-1984	H	-	-	-	+	?
Cladonia coniocraea	22OCHRO	Azores	7-VIII-2003	H	-	-	-	+	?
Cladonia coniocraea	23CONI	India	30-V-2000	H	-	-	-	+	?
Cladonia coniocraea	23OCHRO	Thailand	1-V-2005	H	-	+	-	+	?
Cladonia coniocraea	24CONI	Turkey	V-2005	H	-	-	-	+	?
Cladonia coniocraea	25OCHRO	Taiwan	2001	H	-	-	-	+	?
Cladonia coniocraea	3CONI	Spain	10-XII-1995	MACB 93810	-	-	-	+	?
Cladonia coniocraea	3OCHRO	Spain	6-VIII-2003	MACB 93813	-	+	-	+	?
Cladonia coniocraea	4OCHRO	Spain	21-VII-2006	MACB 95427	JN811374	JN811407	JN811340	JN811440	?
Cladonia coniocraea	5OCHRO	Portugal	2-V-2007	MACB 94635	JN811375	JN811408	JN811341	JN811441	?
Cladonia coniocraea	6CONI	Spain	14-XI-2003	MACB 89744	-	-	-	+	?
Cladonia coniocraea	6OCHRO	Finland	20-VI-1990	MACB 50785	-	-	+	+	?
Cladonia coniocraea	7CONI	Spain	12-X-2002	MACB 93595	-	-	-	+	?

Species	Sample	Country	Date	Voucher	ITS rDNA	*rpb2*	IGS rDNA	*ef1α*	*cox1*
Cladonia coniocraea	8CONI	United Kingdon	13-VI-2002	MACB 94411	-	-	-	+	?
Cladonia coniocraea	8OCHRO	Chile	23-I-2005	MACB 91999	-	-	+	+	?
Cladonia coniocraea	9OCHRO	Canary Islands	16-VI-2007	MACB 102853	-	-	-	+	?
Cladonia conista	13HUMIL	Finland	13-IX-2003	H	JF926629	JF926572	+	-	?
Cladonia conista	14HUMIL	Finland	13-X-2002	H	JF926632	-	+	JF926594	?
Cladonia conista	18HUMIL	Russia	8-VIII-2006	H	JF926619	JF926573	-	JF926595	?
Cladonia conista	1CONIST	USA	29-IX-2006	H	JF926633	JF926568	-	JF926590	?
Cladonia conista	2HUMIL	Spain	23-VII-2000	MACB 92796	JF926613	JF926567	+	JF926589	?
Cladonia conista	3CONIST	USA	6-II-2009	H	JF926635	JF926570	+	JF926592	?
Cladonia conista	11HUMIL	Spain	30-I-2005	MACB 92033	JF926631	-	+	-	?
Cladonia conista	1HUMIL	Chile	11-IV-2003	MACB 97591	JF926612	JF9265066	+	JF926588	?
Cladonia conista	2CONIST	USA	21-IX-2003	H	JF926634	JF9265069		JF926591	?
Cladonia conista	4CONIST	USA	22-IV-2008	H	JF926636	JF9265071	+	JF926593	?
Cladonia cornuta	1CORN	Chile	30-I-2005	MACB 92203	+	-	-	+	?
Cladonia cornuta	4CORN	Canada	14-VIII-2007	H	JN811403	JN811434	JN811373	+	?
Cladonia cornuta	5CORN	Canada	24-V-2004	H	-	-	+	-	?
Cladonia cornuta	6CORN	Finland	19-XI-2006	H	+	-	+	+	?
Cladonia cornuta	7CORN	Finland	4-IX-2008	H	JN811383	JN811426	JN811348	+	?
Cladonia cornuta	9CORN	Finland	4-VIII-2008	H	+	-	-	+	?
Cladonia cornuta	10CORN	Finland	16-X-2009	MACB 101646	JN811385	JN811428	JN811350	-	?
Cladonia cornuta	2CORN	Spain	7-IX-2003	MACB 94344	JN811404	JN811433	JN811371	+	?
Cladonia corsicana	SP1	Spain	23-IV-2006	MACB 100763	JF288797	JF288833	+	?	?
Cladonia corsicana	SP2	Portugal	9-I-2004	MACB 101074	JF288798	JF288834	+	?	?
Cladonia corsicana	SP3	Portugal	9-XII-2006	MACB 101073	JF288799	JF288835	+	?	?
Cladonia corsicana	SP4	Portugal	9-I-2004	MACB 100764	-	JF288836	-	?	?
Cladonia corsicana	SP5	Spain	21-IX-2004	MACB 100765	JF288800	JF288837	+	?	?
Cladonia corymbescens	1CORYM	Thailand	29-VIII-2005	H	-	-	+	?	?
Cladonia cyathomorpha	1CYATH	Spain	15-V-2003	MACB 97543	+	+	+	+	?

Species	Sample	Country	Date	Voucher	ITS rDNA	*rpb2*	IGS rDNA	*ef1α*	*cox1*
Cladonia cyathomorpha	9MONO	Belgium	28-IV-2007	F	+	-	+	+	?
Cladonia cyathomorpha	3CYATH	Norway	10-V-2009	H	+	+	+	+	?
Cladonia cyathomorpha	3MONO	Portugal	2-V-2007	MACB 101278	+	+	+	+	?
Cladonia cyathomorpha	6PYXI	Spain	21-X-1994	MACB 101279	+	+	-	-	?
Cladonia cyathomorpha	CORT	Spain	22-VIII-2001	MACB 97180	+	+	+	+	?
Cladonia ecmocyna	5MACRO	Spain	6-IX-2003	MACB 101650	JN811397	JN811424	JN811352	?	?
Cladonia ecmocyna	1ECMO	Norway	6-VIII-2000	H	JN811399	JN811423	JN811365	?	?
Cladonia ecmocyna	2ECMO	Greenland	23-VI-2004	H	-	-	+	?	?
Cladonia ecmocyna	3ECMO	USA	25-IV-2007	H	-	-	+	?	?
Cladonia ecmocyna	6MACRO	Spain	30-V-2006	MACB 101649	JN811398	JN811425	JN811353	?	?
Cladonia firma	11FIR	Spain	10-VIII-1998	MACB 91615	FM205909	FM207577	?	?	FM208168
Cladonia firma	1FIR	Spain	23-VIII-2003	MACB 91619	FM205907	FM207568	?	+	FM208153
Cladonia firma	3FIR	Spain	9-IV-2004	MACB 60669	FM205908	-	?	?	FM208167
Cladonia firma	7FIR	Spain	5-VI-2004	MACB 90655	FM205910	FM207576	?	?	HQ340075
Cladonia foliacea	4CON	Spain	21-IX-2004	MACB 90565	FM205888	FM207587	?	?	FM208165
Cladonia foliacea	7CON	Portugal	8-VIII-2003	MACB 90517	FM205891	-	?	?	-
Cladonia foliacea	11FOL	Spain	23-VIII-2003	MACB 90533	FM205896	-	?	?	FM208158
Cladonia foliacea	11INT	Spain	3-VII-1996	MACB 90613	FM205902	-	?	?	FM208152
Cladonia foliacea	12FOL	Spain	23-VIII-2003	MACB 90533	FM205897	FM207565	?	?	FM208144
Cladonia foliacea	13CON	Spain	14-V-1991	MACB 41493	FM205892	-	?	?	-
Cladonia foliacea	13INT	Spain	11-I-2006	MACB 92726	FM205903	FM207585	?	?	FM208166
Cladonia foliacea	15INT	Spain	12-I-2006	MACB 92725	FM205924	-	?	?	FM208176
Cladonia foliacea	16FOL	Portugal	10-I-2004	MACB 90503	FM205898	FM207566	?	?	FM208145
Cladonia foliacea	16INT	Spain	12-I-2006	MACB 92725	FM205925	-	?	?	FM208177
Cladonia foliacea	17FOLR	Portugal	6-IX-2006	MACB 95599	FM205914	FM207570	?	?	FM208159
Cladonia foliacea	19CON	Spain	12-X-2002	MACB 91687	FM205893	FM207574	?	?	FM208173
Cladonia foliacea	1CON	Spain	14-V-2004	MACB 90565	FM205886	FM207588	?	?	FM208146
Cladonia foliacea	1FOL	Portugal	10-I-2004	MACB 90506	FM205894	FM207569	?	?	FM208162

Species	Sample	Country	Date	Voucher	ITS rDNA	*rpb2*	IGS rDNA	*ef1α*	*cox1*
Cladonia foliacea	21CONCLA	Spain	3-IX-2002	MACB 90421	FM205918	FM207575	?	?	FM208174
Cladonia foliacea	21FOLR	Scotland	IX-2006	MACB 95602	FM205915	FM2057571	?	?	FM208175
Cladonia foliacea	22FOLCLA	Spain	02-IX-2002	MACB 90414	FM205919	FM2057572	?	?	FM208160
Cladonia foliacea	24FOLCLA	Denmark	9-VII-2001	MACB 95600	FM205920	FM2057573	?	?	FM208161
Cladonia foliacea	25FOL	Finland	9-VII-2001	H	FR695855	HQ340068	?	?	-
Cladonia foliacea	26FOL	Finland	12-X-2003	H	FR695856	HQ340069	?	?	-
Cladonia foliacea	27FOL	Italy	12-X-2003	H	FR695857	HQ340070	?	?	HQ340074
Cladonia foliacea	28FOL	Italy	10-II-2000	H	FR695858	-	?	?	-
Cladonia foliacea	31CON	Sweden	3-VIII-1983	H	FR695859	HQ340064	?	?	-
Cladonia foliacea	32CON	France	12-IV-2007	H	FR695861	HQ340065	?	?	HQ340072
Cladonia foliacea	33CON	Italy	XII-1987	H	FR695860	HQ340065	?	?	-
Cladonia foliacea	34CON	Greece	1-VI-1991	S L65606	FR695862	HQ340067	?	?	HQ340073
Cladonia foliacea	3CON	Spain	21-IX-2004	MACB 90565	FM205887	FM207586	?	?	FM208147
Cladonia foliacea	3FOL	Spain	11-V-2004	MACB 90527	FM205921	-	?	?	-
Cladonia foliacea	3INT	Spain	5-II-2005	MACB 91639	FM205899	FM207583	?	?	FM208149
Cladonia foliacea	5FOL	Spain	22-V-1998	MACB 90574	FM205895	FM207564	?	?	FM208143
Cladonia foliacea	5INT	Spain	27-IX-1999	MACB 90499	FM205900	-	?	?	FM208150
Cladonia foliacea	6CONF	Spain	13-III-2004	MACB 90622	FM205889	FM207567	?	?	FM208148
Cladonia foliacea	6CONG	Spain	13-III-2004	MACB 90622	FM205890	-	?	?	-
Cladonia foliacea	7INT	Spain	20-V-1998	MACB 90440	FM205901	FM207584	?	?	FM208151
Cladonia foliacea	9FOL	Spain	9-IV-2003	MACB 90571	FM205923	-	?	?	FM208164
Cladonia glauca	1GLAU	Spain	26-I-2008	MACB 96751	FN86594	HM243219	?	HM243197	?
Cladonia glauca	3GLAU	Spain	22-VII-2007	MACB 96090	FN86595	HM243220	?	HM243198	?
Cladonia gracilis	15GRAC	Sweden	-	UPS L167919	JN811388	JN811414	JN811356	?	?
Cladonia gracilis	1GRAC	Spain	18-VII-2004	MACB 94216	JN811386	JN811412	JN811354	?	?
Cladonia gracilis	23GRAC	Denmark	13-VIII-2005	H	JN811389	JN811415	JN811357	?	?
Cladonia gracilis	27GRAC	Finland	4-VII-2008	H	JN811395	JN811417	JN811358	?	?
Cladonia gracilis	28GRAC	Finland	18-VIII-2008	H	JN811396	JN811416	JN811359	?	?

Species	Sample	Country	Date	Voucher	ITS rDNA	*rpb2*	IGS rDNA	*ef1α*	*cox1*
Cladonia gracilis	31GRAC	Russia	30-VIII-2008	H	JN811391	JN811418	JN811360	?	?
Cladonia gracilis	32GRAC	Finland	11-IV-2009	H	JN811390	JN811419	JN811361	?	?
Cladonia gracilis	34GRAC	Finland	12-IX-2008	H	JN811392	JN811420	JN811362	?	?
Cladonia gracilis	39GRAC	Finland	16-X-2009	H	JN811394	JN811422	JN811364	?	?
Cladonia gracilis	10GRAC	Scotland	27-VII-2004	L 0753022	-	-	+	?	?
Cladonia gracilis	11GRAC	Sweden	26-VII-1998	L 0753024	-	-	+	?	?
Cladonia gracilis	13GRAC	Austria	28-V-2003	UPS L135110	-	-	+	?	?
Cladonia gracilis	15GRAC	Sweden	15-IX-2007	UPS L167919	-	-	+	?	?
Cladonia gracilis	17GRAC	New Zealand	I-1999	CAMB 565482	-	-	+	?	?
Cladonia gracilis	18GRAC	New Zealand	XII-1998	CAMB 565478	-	-	+	?	?
Cladonia gracilis	19GRAC	New Zealand	I-1999	FH	-	-	+	?	?
Cladonia gracilis	24GRAC	Russia	11-VIII-2005	H	-	-	+	?	?
Cladonia gracilis	25GRAC	Sweden	17-IV-2005	H	-	-	+	?	?
Cladonia gracilis	26GRAC	Denmark	30-X-2003	H	-	-	+	?	?
Cladonia gracilis	29GRAC	USA	27-IV-2002	H	-	-	+	?	?
Cladonia gracilis	30GRAC	Russia	20-X-2003	H	-	-	+	?	?
Cladonia gracilis	33GRAC	Finland	25-V-2005	H	-	-	+	?	?
Cladonia gracilis	35GRAC	Finland	18-IV-2008	H	-	-	+	?	?
Cladonia gracilis	38GRAC	Finland	23-VII-2009	H	JN811393	JN811421	JN811363	?	?
Cladonia gracilis	3GRAC	Argentina	15-III-2005	MA-lichen 16116	-	-	+	?	?
Cladonia gracilis	4GRAC	Chile	18-I-2005	MACB 92148	-	-	+	?	?
Cladonia gracilis	5GRAC	Finland	19-VI-1990	MACB 50709	-	-	+	?	?
Cladonia gracilis	7GRAC	Portugal	8-IX-2006	MACB 94133	-	-	+	?	?
Cladonia gracilis	8GRAC	Spain	23-VIII-2003	MACB 95195	JN811387	JN811413	JN811355	?	?
Cladonia gracilis	9GRAC	Finland	19-IX-1992	MACB 50778	-	-	+	?	?
Cladonia hammeri	1775	USA	22-VI-2000	F	+	+	+	+	?
Cladonia hammeri	18PYXI	USA	VII-2008	H	+	+	+	+	?
Cladonia hammeri	19PYXI	USA	VII-2008	H	+	+	+	+	?

Species	Sample	Country	Date	Voucher	ITS rDNA	*rpb2*	IGS rDNA	*ef1α*	*cox1*
Cladonia hammeri	2NASHI	USA	16-V-2006	H	+	+	-	+	?
Cladonia hammeri	3NASHI	USA	18-X-2006	H	+	+	+	+	?
Cladonia hammeri	6NASHI	USA	2-V-2009	UCR 204973	+	+	+	+	?
Cladonia hammeri	7HAMMER	USA	22-VI-2000	H	+	+	-	+	?
Cladonia hammeri	7NASHI	USA	6-VI-2006	UCR 41270	+	-	-	+	?
Cladonia hammeri	CLCAL1	USA	VII-2008	H	+	+	+	+	?
Cladonia hammeri	CLCAL10	USA	VII-2008	H	+	+	+	+	?
Cladonia hammeri	CLCAL3	USA	VII-2008	H	+	+	+	+	?
Cladonia hammeri	CLCAL9	USA	VII-2008	H	+	+	+	+	?
Cladonia hammeri	5NASHI	USA	6-VI-2006	UCR 41269	+	+	+	+	?
Cladonia hammeri	CLCAL4	USA	VII-2008	H	+	+	+	+	?
Cladonia hammeri	CLCAL7	USA	VII-2008	H	+	+	+	+	?
Cladonia humilis	10HUMIL	Spain	11-I-2006	MACB 92803	JF926628	JF926581	+	JF926603	?
Cladonia humilis	15HUMIL	Madeira	8-II-2004	H	JF926616	JF926582	+	JF926604	?
Cladonia humilis	16HUMIL	Turkey	VI-2005	H	JF926617	JF926577	+	JF926605	?
Cladonia humilis	4HUMIL	Spain	19-II-2005	MACB 92885	JF926625	JF926584	+	JF926597	?
Cladonia humilis	4NASHI	Spain	5-II-1998		+	-	-	+	?
Cladonia humilis	5HUMIL	Spain	5-XI-2004	MACB 92807	JF926622	JF926585	+	JF926598	?
Cladonia humilis	5KUROK	Taiwan	13-X-2001	H	+	+	-	+	?
Cladonia humilis	6HUMIL	Portugal	9-XII-2006	MACB 97326	JF926626	JF926587	+	JF26599	?
Cladonia humilis	7HUMIL	Portugal	10-I-2004	MACB 92818	JF926627	JF926583	+	JF26600	?
Cladonia humilis	8HUMIL	Spain	14-I-2007	MACB 95913	JF926614	JF926575	+	JF26601	?
Cladonia humilis	9HUMIL	Spain	4-IX-2007	MACB 95931	JF926615	JF926576	+	JF26602	?
Cladonia humilis	CLCAL5	USA	VII-2008	H	JF926623	JF926586	+	JF26609	?
Cladonia humilis	CLCAL8	USA	VII-2008	H	JF926624	JF926574	+	JF26610	?
Cladonia humilis	17HUMIL	USA	21-X-2005	H	JF926618	JF926578	+	JF26606	?
Cladonia humilis	19HUMIL	USA	12-VII-2008	H	JF926620	JF926579	+	JF26607	?
Cladonia humilis	20HUMIL	Croatia	31-III-2010	MACB 101103	JF926621	JF926580	-	JF26608	?

Species	Sample	Country	Date	Voucher	ITS rDNA	*rpb2*	IGS rDNA	*ef1α*	*cox1*
Cladonia latiloba	1LATEO	Brasil	26-VII-1999	H	JN621937	JN621967	-	JN621998	?
Cladonia macroceras	11MACRO	China	2-VII-2000	FH	-	-	+	?	?
Cladonia macroceras	13MACRO	Russia	28-VIII-2000	H	-	-	+	?	?
Cladonia macroceras	1MACRO	Spain	17-VIII-2006	MACB 94199	-	-	+	?	?
Cladonia macroceras	2MACR	Andorra	19-VIII-2006	MACB 94200	JN811382	JN811411	JN811351	?	?
Cladonia macroceras	8MACRO	Austria	24-VII-1994	L 0753010	-	-	+	?	?
Cladonia magyarica	2MAGY	Hungary	-	MACB 98243	+	+	+	+	?
Cladonia magyarica	4MAGY	Ukranie	17-IV-2006	H	+	+	+	+	?
Cladonia maxima	1MAXI	Russia	4-VIII-2006	H	-	-	+	?	?
Cladonia nashii	1NASHI	USA	18-X-2006	H	+	+	-	-	?
Cladonia nashii	5HAMMER	Mexico	25-XI-1999	H	+	+	-	-	?
Cladonia nashii	CLCAL2	USA	VII-2008	H	+	+	+	+	?
Cladonia nashii	1FIMB	USA	25-V-2000	H	+	+	+	+	?
Cladonia pulvinata	4PUL	Spain	19-VI-1995	MACB 91646	FM205911	FM207579	+	?	FM208172
Cladonia pulvinata	5PULR	Portugal	8-IX-2006	MACB 95597	FM205912	FM207580	+	?	FM208155
Cladonia pulvinata	7PULR	Portugal	8-IX-2006	MACB 94339	FM205917	FM207581	-	?	FM208156
Cladonia pulvinata	9PULR	Spain	20-VII-2006	MACB 95598	FM205913	FM207582	-	?	FM208157
Cladonia rangiformis	10RANG	Turkey	20-VIII-2005	H	+	+	+	?	?
Cladonia rangiformis	12RANG	Iran	10-V-2003	H	+	+	+	?	?
Cladonia rangiformis	14RANG	Finland	20-IX-2005	H	+	+	+	?	?
Cladonia rangiformis	3RANG	Madeira	7-X-2005	H	+	+	+	?	?
Cladonia rangiformis	4RANG	Canary Islands	12-IV-2006	H	-	+	-	?	?
Cladonia rangiformis	6RANG	Sweden	30-V-2000	H	JN811401	JN811430	JN811368	?	?
Cladonia rangiformis	8RANG	Greece	11-X-2008	H	+	+	+	?	?
Cladonia rangiformis	9RANG	Greece	3-X-2008	H	+	+	+	?	?
Cladonia rangiformis	11RANG	Iran	3-I-2004	H	+	+	+	?	?
Cladonia rangiformis	13RANG	Azores	29-VII-2001	H	+	+	+	?	?
Cladonia rangiformis	2RANG	Spain	22-I-2009	H	+	+	+	?	?

Species	Sample	Country	Date	Voucher	ITS rDNA	*rpb2*	IGS rDNA	*ef1α*	*cox1*
Cladonia rangiformis	5RANG	Netherlands	4-IX-2002	H	JN811400	JN811429	JN811367	?	?
Cladonia rangiformis	7RANG	Greece	29-IV-2002	H	+	+	+	?	?
Cladonia rangiformis	15RANG	Canary Islands	8-X-1999	MACB 90888	-	+	+	?	?
Cladonia rangiformis	1RANG	Spain	28-XI-2007	MACB 96193	JF288803	JF288838	JN811366	?	?
Cladonia rei	15REI	Netherlands	III-2009	Aproot 68588	FN86590	HM243207	?	HM243193	?
Cladonia rei	16REI	Japan	30-XI-2007	UPS L170710	FN86593	-	?	-	?
Cladonia rei	17REI	Czech Republic	18-IV-2009	J. Vondrák 7024	FN86587	HM243208	?	HM243194	?
Cladonia rei	18REI	Czech Republic	2-IV-2009	J. Vondrák 6967	FN86588	HM243209	?	HM243195	?
Cladonia rei	19REI	Czech Republic	4-IV-2009	J. Vondrák 7026	FN86589	-	?	HM243196	?
Cladonia rei	2REI	Canada	5-VIII-2002	S L58841	FN86580	HM243200	?	HM243185	?
Cladonia rei	3REI	Sweden	2-VIII-2004	S F52894	FN86581	HM243201	?	HM243186	?
Cladonia rei	4REI	Norway	3-IX-2008	BG L86605	FN86582	HM243202	?	HM243187	?
Cladonia rei	5REI	Canada	7-IX-2007	BG L86394	FN86583	HM243203	?	HM243188	?
Cladonia rei	6REI	USA	10-VIII-2004	S F53070	FN86584	HM243204	?	HM243189	?
Cladonia rei	7REI	Spain	15-IX-2007	MACB 92216	FN86585	HM243205	?	HM243190	?
Cladonia rei	8REI	Spain	16-VIII-2006	MACB 100473	FN86586	HM243206	?	HM243191	?
Cladonia rei	11REI	Slovakia	17-V-1988	BRA-CR 10005	FN86591	-	?	HM243192	?
Cladonia rei	12REI	Czech Republic	VIII-1986	BRA-CR 10044	FN86592	-	?	-	?
Cladonia sp.1	10SYMP	Austria	26-V-2000	UPS L135579	JN621927	JN621960	?	JN621991	?
Cladonia sp.1	13SYMP	Ukranie	6-V-2005	H	JN62130	JN621963	?	JN621994	?
Cladonia sp.1	1CARI	Spain	18-VIII-2006	MACB 94205	FR69563	HQ340071	?	JN621904	HQ340075
Cladonia sp.1	2CARI	Spain	6-IX-2006	MACB 93984	JN621906	JN621938	?	JN621970	?
Cladonia sp.1	4SYMP	Spain	11-V-1998	MACB 92737	JN621921	JN621954	?	JN621986	?
Cladonia sp.1	6CARI	Spain	5-XI-2006	MACB 93018	JN621910	JN621942	?	JN621974	?
Cladonia sp.1	7CARI	Spain	22-V-2004	MACB 92995	JN621911	JN621943	?	JN621975	?
Cladonia sp.2	12SYMP	Russia	24-VIII-1997	H	JN621929	JN621962	?	JN621993	?
Cladonia subcariosa	2SUBCARI	USA	17-VII-2002	H	JN621935	JN621968	?	JN621999	?
Cladonia subcariosa	1SUBCARI	USA	8-VII-2003	H	JN621936	JN621969	?	JN622000	?

Species	Sample	Country	Date	Voucher	ITS rDNA	*rpb2*	IGS rDNA	*ef1α*	*cox1*
Cladonia subconistea	1SUBCONI	North Korea	25-III-2003	H	+	+	+	+	?
Cladonia subconistea	2KUROK	Japan	18-XI-2002	H	+	+	+	+	?
Cladonia subconistea	3KUROK	China	21-X-2002	H	+	+	-	+	?
Cladonia subconistea	55724	China	7-X-1998	H	AF455207	+	+	+	?
Cladonia subconistea	1KUROK	Japan	22-X-2002	H	+	-	+	-	?
Cladonia subconistea	55878	China	11-X-1998	H	AF455210	+	+	+	?
Cladonia subturgida	1SUBT	Spain	28-IV-1993	MACB 49934	JF288791	-	?	?	?
Cladonia subturgida	3IBER	Spain	20-VII-2006	MACB 100441	JF288788	JF288828	?	?	?
Cladonia subturgida	4IBER	Spain	3-V-2005	MACB 100442	JF288789	JF288829	?	?	?
Cladonia subturgida	1IBER	Portugal	5-IX-2006	MACB 93695	JF288786	JF288826	?	?	?
Cladonia subturgida	2IBER	Spain	6-II-2003	MACB 93537	JF288787	JF288827	?	?	?
Cladonia subturgida	2SUBT	Spain	24-IX-2004	MACB 100445	JF288792	JF288823	?	?	?
Cladonia subturgida	3SUBT	Spain	6-II-2003	MACB 99488	JF288793	JF288824	+	+	?
Cladonia subturgida	4SUBT	Spain	1-V-2007	MACB 100447	JF288794	JF288825	?	?	?
Cladonia subturgida	5IBER	Spain	6-IX-2003	MACB 100443	JF288790	JF288830	?	?	?
Cladonia subturgida	7IBER	Spain	16-VI-2007	MACB 99466	JF288797	JF288831	?	?	?
Cladonia subturgida	TYPEIBER	Spain	16-VI-1993	MACB 49936	JF288795	JF288832	?	?	?
Cladonia subulata	2SUBU	Spain	9-XI-2003	MACB 93837	FN86567	HM243211	+	HM243175	?
Cladonia subulata	4SUBU	Sweden	24-VII-2007	S F90966	FN86569	HM243213	?	HM243177	?
Cladonia subulata	6SUBU	Spain	18-VI-2004	MACB 95159	FN86577	-	?	-	?
Cladonia subulata	7SUBU	Spain	8-XII-2007	MACB 96350	FN86571	HM243215	?	HM243179	?
Cladonia subulata	13SUBU	Netherlands	III-2009	L. Spier	FN86573	HM243217	?	-	?
Cladonia subulata	15SUBU	France	13-VII-2000	L 75293	FN86579	-	?	-	?
Cladonia subulata	16SUBU	Czech Republic	2-IV-2009	Vondrák 6983	FN86574	-	?	HM243182	?
Cladonia subulata	18SUBU	Denmark	14-III-2009	J. Vondrák 6967	FN86575	-	?	HM243183	?
Cladonia subulata	19SUBU	Austria	26-IV-2009	F. Berger 23733	FN86576	HM243218	?	HM243184	?
Cladonia subulata	1SUBU	Spain	22-VII-2006	MACB 93151	FN86566	HM243210	+	HM243174	?
Cladonia subulata	3SUBU	Sweden	15-VII-2005	S F52879	FN86568	HM243212	?	HM243176	?

Species	Sample	Country	Date	Voucher	ITS rDNA	*rpb2*	IGS rDNA	*ef1α*	*cox1*
Cladonia subulata	5SUBU	Spain	13-III-2008	MACB 97275	FN86570	HM243214	?	HM243178	?
Cladonia subulata	8SUBU	Portugal	5-IX-2006	MACB 93692	FN86572	HM243216	+	HM243180	?
Cladonia subulata	12SUBU	Slovakia	8-V-1971	BRA-CR 10048	-	-	?	HM243181	?
Cladonia subulata	9SUBU	Chile	26-I-2005	MACB 92216	FN86578	-	?	-	?
Cladonia symphycarpa	10CARI	Norway	6-VIII-2006	BG L784035	JN621914	JN621946	?	JN621978	?
Cladonia symphycarpa	14SYMP	Bosnia and Herzegobina	28-III-2010	MACB 101124	JN621931	JN621964	?	JN621995	?
Cladonia symphycarpa	1SYMP	Spain	25-VII-2006	MACB 93496	JN621918	JN621951	?	JN621982	?
Cladonia symphycarpa	2SYMP	Spain	12-X-2002	MACB 93559	JN621919	JN621952	?	JN621983	?
Cladonia symphycarpa	6SYMP	Sweden	28-VIII-2002	S L50055	JN621923	JN621956	?	JN621988	?
Cladonia symphycarpa	7SYMP	USA	15-VII-2004	S F53075	JN621924	JN621957	?	JN621989	?
Cladonia symphycarpa	8SYMP	Germany	1-V-2003	B 60 0122320	JN621925	JN621958	?	JN621990	?
Cladonia symphycarpa	9SYMP	Germany	7-VIII-1994	B 60 0125267	JN621926	JN621959	?	JN621984	?
Cladonia thomsonii	1THOMS	Russia	15-VII-1996	H	JN811402	JN811431	JN811369	+	?
Cladonia turgida	1TURG	Canada	18-VI-2006	H	JF288801	+	+	+	?
Cladonia turgida	2TURG	Canada	21-IX-2008	H	JF288802	+	+	+	?

Table 2S. Primers used to amplify ITS rDNA, IGS rDNA, *rpb2*, *ef1α* and *cox1*.

Primer name	Region	Sequence (5'-3')
ITS1F	ITS	cttggtcatttagaggaagtaa
ITS4	ITS	tcctccgcttattgatatgc
RPB2-5F	*rpb2*	gaygayngwgatcayttygg
RPB2-7R	*rpb2*	cccatrgcttgyttrcccat
CLRPB2-5F	*rpb2*	ctgtttcgaacgctgtttca
CLRPB2-7R	*rpb2*	cgcatccacgtattcaacaa
RPB2dRaq	*rpb2*	gctgctaagtctaccat
RPB2rRaq	*rpb2*	atcatgcttggaatctc
IGSf	IGS	tagtggccgwtrgctatcatt
IGSr	IGS	tgcatggcttaatctttgag
CLEF3F	*ef1α*	ggcaaaggctccttcaagt
CLEF3R	*ef1α*	gccaataccaccgatcttgt
5959F	*cox1*	tcttaacgttgctgtatgctg
6711R	*cox1*	gaaccgaaactagtagaaccata

ANEXO 3: Iconografía

Lámina 1: A) *C. cenotea*, B) *C. cervicornis*, C) *C. coniocraea*, D) *C. cornuta* subsp. *cornuta*, E) *C. ecmocyna* F) *C. farinacea*.

Lámina 2: A) *C. firma*, B) *C. foliacea*, C) *C. furcata*, D) *C. glauca*, E) *C. gracilis* subsp. *gracilis* F) *C. gracilis* subsp. *elongata* G) *C. gracilis* subsp. *tenerrima*.

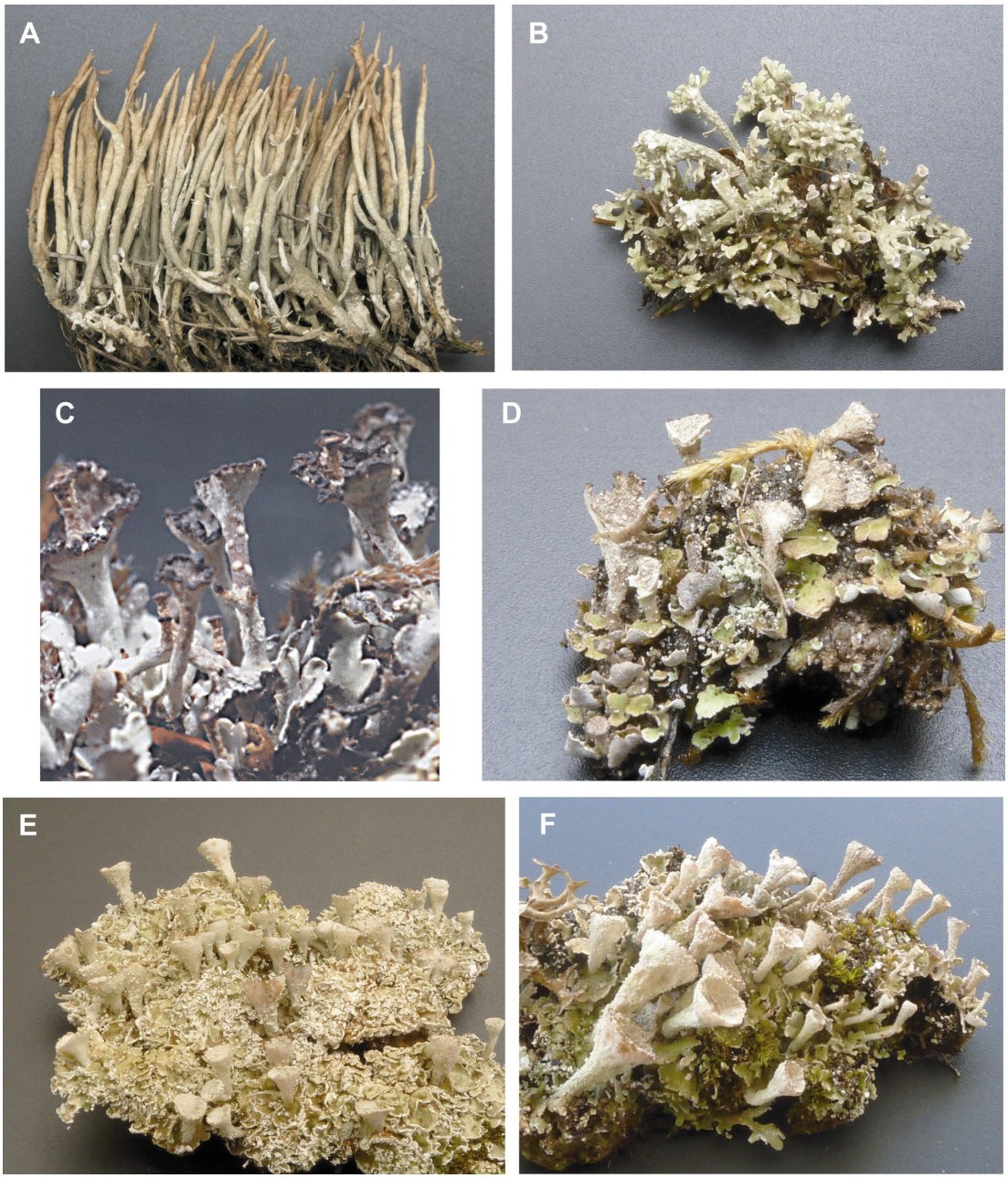

Lámina 3: A) *C. macroceras*, B) *C. magyarica*, C) *C. pulvinata*, D) *C. pulvinella*, E) *C. pocillum* F) *C. pyxidata*.

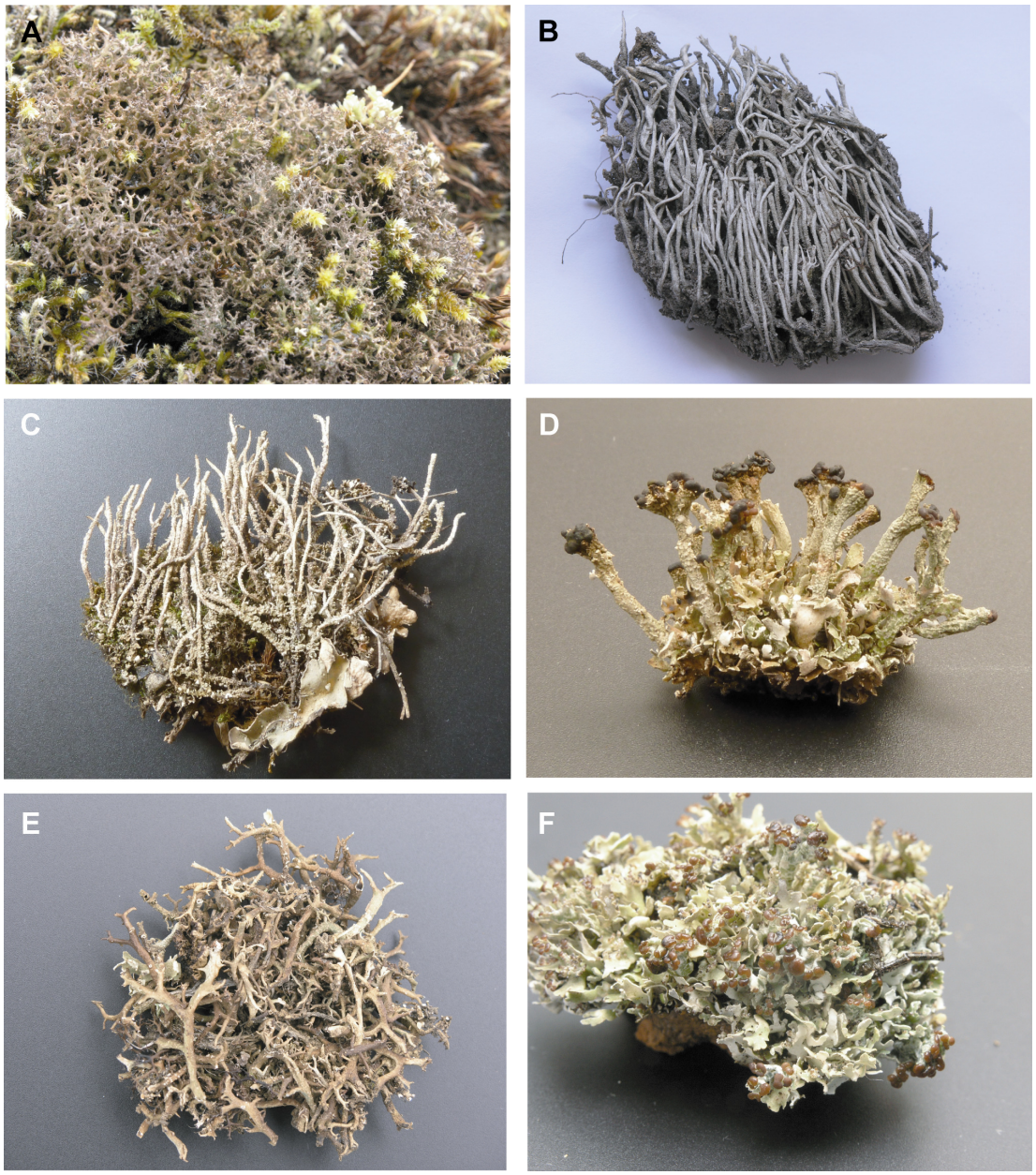

Lámina 1: A) *C. rangiformis*, B) *C. rei*, C) *C. scabriuscula*, D) *C. subcariosa*, E) *C. subrangiformis* F) *C. subturgida*.

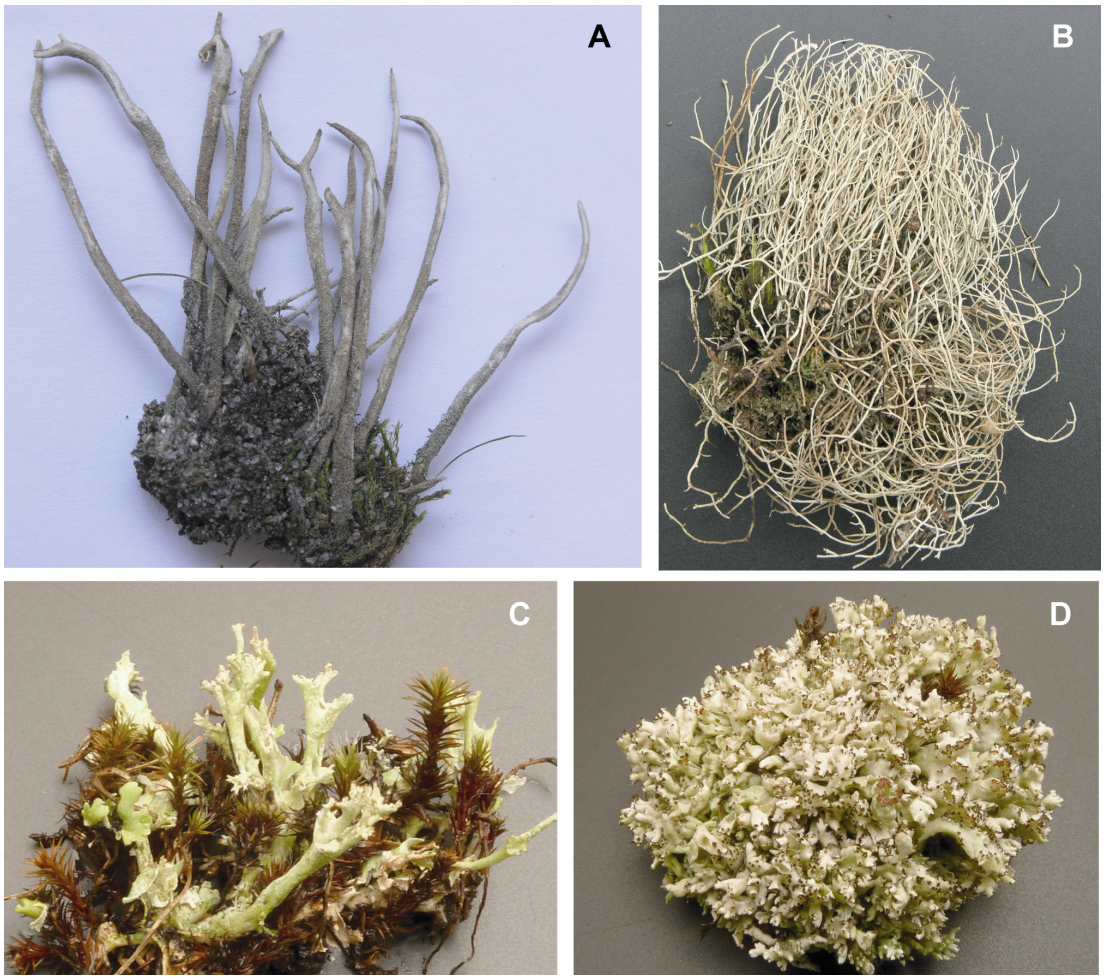

Lámina 5: A) *C. subulata*, B) *C. stereoclada*, C) *C. turgida*, D) *Pycnothelia papillaria*